# Abstract

Ekelund, A. 2003. Phosphorus and the dairy cow - influence of intake level, source and stage of lactation on apparent digestibility and bone turnover. Doctoral thesis.
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The general aim of the present work was to investigate phosphorus balance in the dairy cow, with reference to the amount and source of phosphorus. Furthermore, biochemical bone markers were used to study the bone turnover during the lactation and dry period.

Phosphorus is located in every cell of the body and has more known functions than any other mineral element in the animal body. Phosphorus is also an important constituent of milk, and is therefore required in large amounts in a high yielding dairy cow. It is of great nutritional, but also environmental and economical importance, to meet the animals' phosphorus requirement, and yet to neither feed more or less than necessary is a complicated balance.

Three experiments were included in this thesis. In the first experiment five cows received, at different times, five levels of P intake, from 44 to 142 g/day, in a Latin Square Design. In the second experiment four cows received four different concentrates with various phosphorus sources; rapeseed, sunflower seed/palm kernel, wheat middlings/bran and monosodium phosphate, also in a Latin Square Design. The third study included 21 cows and covered a dry period, a lactation, and the following dry period. Two different levels of phosphorus, 0.32% and 0.43% of DM, were fed during the first four months of the lactation. From the fifth month until the end of the lactation one level, 0.43% of DM, were fed.

In all studies, total collections of faeces were conducted for five consecutive days during each collection period. Milk yield and feed intake were also recorded. During the third experiment blood samples were taken and analysed for osteocalcin and CTx. Osteocalcin is a specific product of the osteoblast and was therefore used as a measurement of bone formation. CTx is a specific product of the osteoclast and was therefore used as a measurement of bone resorption.

The results presented here show that increased phosphorus intake decreased the apparent digestibility of phosphorus linearly. There was a considerable variation in faecal P excretion between days, mostly due to differences in the daily amounts of faecal DM. The apparent digestibility of phosphorus did not differ between the P sources investigated. Cows receiving 0.32% phosphorus of DM excreted less phosphorus in the faeces and had a higher apparent digestibility of phosphorus than cows receiving 0.43%, but there were no differences in phosphorus retention between the groups. The bone markers showed an appreciable increase in bone resorption immediately after parturition, whilst bone formation was highest during mid lactation.

Phosphorus fed in excess of the requirements was excreted in the faeces, showing the pointlessness of feeding phosphorus above the needs of the cow. The large variation in faecal phosphorus output between days showed the necessity of total faeces collections when studying phosphorus balance. It was suggested that the naturally occurring bone resorption in early lactation could be taken into account in diet calculations, leading to lower P intake during this time, and consequently less P excretion in faeces.

*Keywords*: apparent digestibility, biochemical bone marker, bone turnover, dairy cow, feed stuff, inorganic phosphorus, phosphorus excretion, phosphorus requirement

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What goes in must come out

# Contents

# Appendix

## Papers I-IV

The present thesis is based on the following papers, which will be referred to by their Roman numerals:

**I** Ekelund, A., Spörndly, R., Valk, H. & Murphy, M. 2003. Effects of varying monosodium phosphate intake on phosphorus excretion in dairy cows. *Submitted*.

**II** Ekelund, A., Spörndly, R., Valk, H. & Murphy, M. 2003. Influence of feeding various phosphorus sources on apparent digestibility of phosphorus in dairy cows. *Animal Feed Science and Technology 109 (In press)*.

**III** Holtenius, K. & Ekelund, A. 2003. Biochemical markers of bone turnover in the dairy cow during lactation and in the dry period. *Submitted*.

**IV** Ekelund, A., Spörndly, R. & Holtenius, K. 2003. Influence of low phosphorus intake during early lactation on apparent digestibility of phosphorus and bone metabolism in dairy cows. *Manuscript*.

# List of abbreviations

| | |
|---|---|
| **ADP** | Apparent digestibility of phosphorus |
| **BW** | Body weight |
| **Ca** | Calcium |
| **CTx** | C-telopeptide fragments of collagen type I |
| **DM** | Dry matter |
| **DMI** | Dry matter intake |
| **DOM** | Digestible organic matter |
| **ECM** | Energy corrected milk |
| **LP** | Diet with low phosphorus content |
| **LSM** | Least squares means |
| **ME** | Metabolisable energy |
| **MF** | Mineral feed |
| **MJ** | Megajoule |
| **MSP** | Monosodium phosphate |
| **NDF** | Neutral detergent fiber |
| **NP** | Diet with normal phosphorus content |
| **OC** | Osteocalcin |
| **OM** | Organic matter |
| **P** | Phosphorus |
| **PTH** | Parathyroid hormone |
| **RS** | Diet with rape seed |
| **SED** | Standard error of the difference |
| **SLU** | Swedish University of Agricultural Sciences |
| **SRB** | Swedish Red and White Breed |
| **SSP** | Diet with sunflower seed and palm kernel |
| **TAC** | True absorption coefficient |
| **WMB** | Diet with wheat middlings and bran |

# Background

The element phosphorus, also known as the Devils' Element, was discovered by alchemists in the seventeenth century. Many alchemists have laid claim to its discovery, but according to Emsley (2000) it probably was Hennig Brandt, an alchemist from Hamburg. He was experimenting with urine to find the philosophers' stone when something strange began to happen. Glowing fumes, which had pungent garlic-like odour, filled the vessel and from the end of the retort shining liquid was dripping and bursting into flames. He caught this liquid in a glass vessel and found that it solidified but continued to gleam. This explains the word phosphorus; derived from the ancient Greek words *phos* meaning "light" and *phorous* meaning "bringing". In 1769, the Swedish chemists Scheele and Gahn found that phosphorus is an important constituent of bones. They later isolated phosphorus from bone ash and produced phosphoric acid by the action of nitric acid on phosphorus. The reaction of the bones with nitric or sulphuric acid produced phosphoric acid that, when heated up with coal, produced elementary phosphorus. This was the first method for the commercial production of phosphorus and by the 1800's matches made of phosphorus were in such demand in England that battlefields were being scavenged for human bones to produce the element.

Today, we make use of phosphorus for many purposes. Industrial phosphates are used in modern and efficient detergents and cleaning agents. Phosphoric acid is a versatile corner-stone of inorganic chemistry, which forms the basis for a wide range of industrial chemicals, including fertilisers and feed phosphates, which are important in animal nutrition. Whilst never found occurring freely in nature, phosphorus is widely distributed in combination with other minerals. Phosphate rock, which contains the mineral apatite, an impure tri-calcium phosphate, is an important source of the element. Large deposits of apatite are found in Russia, Morocco, and in the states of Florida, Tennessee, Utah and Idaho in the USA. In Western Europe, the only place where it is profitable to mine apatite is Siilinjärvi in Finland. The Finnish deposits of apatite are of very high quality and only contain small amounts of heavy metals and other undesirable substances. Phosphate rock was first used in 1850 as a source of phosphorus and today phosphorus, phosphates and phosphoric acid are solely obtained from phosphate rock.

Modern dairy production is characterized by high milk yield levels, which have been achieved by genetic and managemental improvements. The yearly milk production per cow, among recorded Swedish cows, has increased by almost 100% during the last 40 years (Swedish Dairy Association, 2003). There are actually some cows that exceed 100 kg ECM per day. This involves increased demands on the precision required in cow nutrition in order to derive advantage from the cows' genetic capacity and to avoid production diseases. The mean phosphorus content in cow milk is about 0.95 g/litre (Paul & Southgate, 1978) and consequently large amounts of phosphorus are excreted in milk in high-yielding dairy cows. In order to sustain milk production, it is important to meet the cows' requirements for dietary phosphorus. Although all regular feedstuffs contain phosphorus, dietary supplements of inorganic feed phosphates are still necessary for modern, high-yielding cows. It is important that we supplement the diets with sufficient phosphorus, as minor or temporary deficiency is difficult to discover. It may also take time before a prolonged deficiency produces symptoms, like decreased feed intake and lower milk yield (Valk et al., 1999). In cases of severe phosphorous deficit, cows may show a craving, appearing as an avid appetite for bones. This may result in botulism due to the ingestion of putrefied carcass debris. The behaviour is widespread in Australia, and other countries, where the soils are phosphorus deficient (Blair-West et al., 1989).

On the other hand, it is also important to not feed more phosphorus than the animal requires. Excess phosphorus is excreted in faeces, and intensive animal production may cause environmental problems, due to phosphorus overload caused by leakage from farms. When the manure applied to agricultural land contains more phosphorus than the crops can take up, there is a risk for phosphorus runoff in surface water. The higher the phosphorus content in fields, the more phosphorus runoff occurs. Excess phosphorus in water can allow algae populations to grow rapidly, or to “bloom”. The subsequent decomposition of the algae consumes dissolved oxygen in the water. The resulting lack of dissolved oxygen, is the major factor affecting the growth and reproduction of fish, clams, crabs, oysters, and other aquatic animal life. An algal bloom and the subsequent decrease in dissolved oxygen is known as eutrophication. Phosphorus is a source of major concern, as it is the main nutrient that limits the production of all biological groups in freshwaters. One problem in using manure as fertilizer is that the ratio between nitrogen and phosphorus in typical manure is roughly half of the optimum for most crops. If manure is applied to fields in sufficient quantities to meet the crops’ nitrogen requirements, there will be a phosphorus over-application and accumulation over time. Therefore, it is of environmental concern to decrease phosphorus excretion in faeces.

Phosphorus is the most expensive macro mineral element supplemented in the diets of dairy cattle. It is therefore of economical importance to formulate precise and accurate diets to meet requirements at the least cost. In addition to the risk of eutrophication, this is a further important reason not to feed inorganic feed phosphates in excess. Also, all natural resources are limited and apatite mining at the present rate cannot continue. The earth has an abundant supply of phosphorus, but many of the deposits are under coastal water and mining this underwater phosphorus would be costly. It is estimated that the current supply of accessible, cheap phosphorus, only will last about another 100 years. For that reason it should be used sparingly. In conclusion, it is of great environmental, economical and nutritional importance to meet the animals’ phosphorus requirement, and neither feed more or less than necessary. These are the reasons for today’s interest in further defining the phosphorus requirements of dairy cattle.

# Introduction

## Functions of phosphorus

Phosphorus (P) is located in every cell of the body and has more known functions than any other mineral element in the animal body. In addition to being of major importance as a constituent of bone, P is an essential component of organic compounds involved in almost every aspect of metabolism. P plays an important part in muscle, energy, carbohydrate, amino acid, fat, and nerve tissue metabolism and phosphate is an important part of the nucleic acids DNA and RNA. It is also a component of many coenzymes and is found in compounds such as adenosine di- and triphosphate.

## Metabolism

Metabolism of P in ruminants is complicated (Figure 1). One reason for this complexity, is that a significant amount of P is incorporated by the rumens' microbial population as a component of their nucleic acids and phospholipids. Furthermore, ruminants secrete a large amount of saliva from the salivary glands into the rumen, more than 100 litres per day. The P concentration in cattle saliva is 370-720 mg/litre. These levels are considerably higher than that of bovine blood plasma, which is about 40-80 mg/litre. Therefore, the salivary glands have an important part to play in the regulation and the homeostasis of P (Clark et al., 1973). About 80-85% of P in the body is found in bones and teeth. Bone is an active tissue that undergoes continuous metabolic changes and is remodelled by the activity of osteoblasts and osteoclasts, which form and resorb bone, respectively. Consequently, there is a continuous P exchange between bone and blood and when dietary P is insufficient, mobilization of stored P becomes important. The excretion of P in monogastric species is mainly in the form of urine. However, in ruminants P excretion occurs mainly via the faeces and it is only under certain conditions that urinary excretion may occur. The P in faeces is either exogenous, which is unabsorbed dietary P (Bromefield & Jones, 1970) or endogenous, which is of

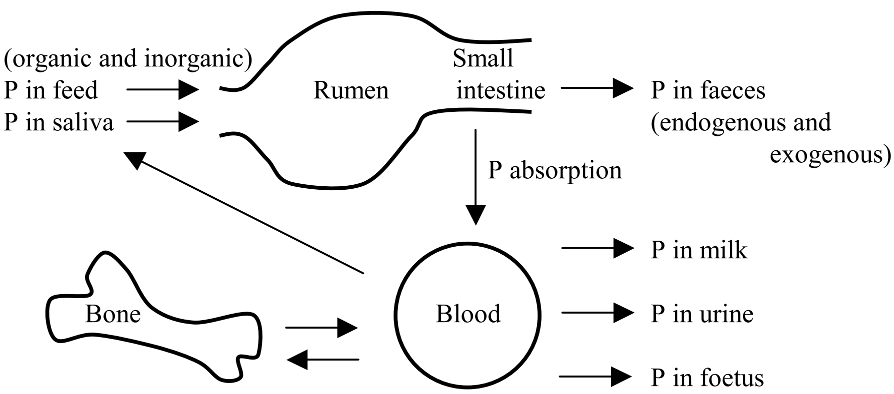


Figure 1. *Phosphorus (P) metabolism in the dairy cow.*

bodily origin, mainly from saliva P (Wadsworth & Cohen, 1976) but there is also P from intestinal cells (Playne, 1976) and digestive secretions.

### *Ruminal microorganisms and saliva*

The total P content of rumen microbes ranges from 2-6% of the DM and the P supply from saliva is important for rumen microbial nutrition (Milton & Ternouth, 1984). Rumen bacteria, protozoa and fungi all have a requirement for P in order to maintain rates of growth and reproduction. Any decrease in these rates will result in a lower level of fibre digestion for the ruminant, with a consequent decrease in feed intake and production. Thus, the microbes have a requirement for P which is separate from the requirements of cattle. There are many publications that report data on the P requirements of ruminal microbes. These data have mainly been obtained using batch-culture systems (Ammerman et al., 1965; Durand, Beumatin & Dumay, 1983; Milton & Ternouth, 1984), and more recently using continuous culture techniques (Komisarczuk et al., 1987). These reports show that when inorganic P levels are less than 50-80 mg/litre, microbial activity is likely to be reduced due to a reduction in cellulose and hemicellulose breakdown (Komisarczuk et al., 1987). According to Komisarczuk-Bony & Durand (1991) the available P supply in the rumen should be at least 5 g P/kg fermented organic matter. The P should be supplied via dietary- and saliva-P for optimal cell wall degradation and protein synthesis. The critical levels of P for adequate microbial fermentation apply to both ruminal and caeco-colic microbes (Milton & Ternouth, 1984). The salivary secretion of P constitute approximately 80% of the endogenous P recycled to the gastrointestinal tract (Care, 1994). Salivary P is inorganic (orthophosphates) and highly available to the microbes. The amount of P secreted via the saliva depends on the DM intake, usually combined with P intake, and the fibre content of the diet. On more fibrous diets, saliva secretion will be increased and, although the P content in the saliva may be decreased, total salivary P secretion will increase. Therefore, diets which stimulate salivary secretion in cattle may improve the utilization of P (Yano, Yano & Breves, 1991). Khorasani et al. (1993) found that cows fed triticale silage, high in neutral detergent fiber (NDF), secreted more salivary P into the rumen than cows fed alfalfa and barley silages, which are lower in NDF. The major source of P flowing into the rumen is not the diet, but the salivary secretions (Tomas, Moir & Somers, 1973). Reducing salivary P during P deficiency and increasing salivary P during P excess, represents an efficient method of P regulation unique to the ruminant.

### *Absorption*

P occurs in plants in either an inorganic (orthophosphate, pyrophosphate) or organic (phospholipids, phosphosugars, ADP/ATP, nucleic acids polymers and phytate) form. Inorganic P sources, which are soluble in water or diluted acids, are available for absorption in ruminants. The solubility of P in organic compounds depends on the ability of the animal to convert P into an inorganic form or by changing the organic P into more acceptable organic forms (Underwood & Suttle, 1999). The absorption of ingested P depends on its solubility at the point of contact with the absorbing membranes (McDowell, 1992). Most of the dietary organic P will be hydrolysed by the microbes into inorganic forms. The remaining organic P, which has not been hydrolysed in the rumen, will be soluble in the low pH of the abomasum (Breves & Schröder, 1991; Care, 1994). The availability of the microbial P may also depend on pH and Playne (1976) has suggested that if the pH is above 6 then the P may be unavailable. The absorption of P is thus favoured by factors, which operate to hold P in solution, and an acid medium tends to prevent the formation of the insoluble, and thus unabsorbable, tricalcium phosphate. The term retention is used to denote the amount of P that remains in the body. The availability of P may be defined as the proportion of the dietary P which may be absorbed by the animal when it is absorbing P at a maximal rate. The current measurements of absorption are the product of the coefficients of availability and absorption. Due to the high amount of P that is recycled in the saliva, salivary secretion to some extent is related to plasma P concentrations and hence to P intake, the dietary absorption coefficients are homeostatic mechanisms that are not easy to interpret.

P is primarily absorbed in the small intestine (Reinhardt, Horst & Goff, 1988), and the absorption is thought to occur mainly in the duodenum and jejunum (Care, Barlet & Abdel-Hafeez, 1980; Scott, McLean & Buchan, 1984). Only small amounts are absorbed from the rumen, omasum, and abomasum, however, little is known about mechanisms and regulation of absorption anterior to the small intestine (Breves & Schröder, 1991). Presumably, P absorption occurs via two distinct mechanisms. A saturable vitamin D dependent active transport system operates when animals are fed low dietary P. Horst (1986) suggests that low plasma P will stimulate 1,25-dihydroxycholecalciferol synthesis. The resulting increase in 1,25-dihydroxycholecalciferol stimulates the intestine to absorb P more efficiently. Passive absorption predominates when normal to large amounts of potentially absorbable P are consumed, and absorption is thought to be related directly to the amount in the lumen of the small intestine and to the concentrations in blood plasma (Wasserman & Taylor, 1976; Care, Barlet & Abdel-Hafeez, 1980). However, according to Braithwaite (1983) there is generally an inverse relationship between P intake and the absorption coefficient. When circulating concentrations of P are adequate, the P absorption rate may be reduced by saturation or inhibition in the absorptive mechanism, and Morse et al. (1992) observed an inverse relationship between P intake and P absorption when non-limiting levels of P were fed. On the other hand, Khorasani et al. (1997) found that the relationship between P intake and total absorption of P was curvilinear, suggesting that forage source, due to the NDF-content, had a greater effect on total P absorption than P intake. The effect of P intake on P absorption needs further investigations.

Factors which may reduce the coefficient of absorption of P are interactions with other minerals such as, Ca, Mg, Mn, K, Fe, Zn, Mo and Al, due to formation of insoluble and, thus, unabsorbable complexes with P. The desirable Ca:P ratio has for a long time been defined as lying between 1:1 and 2:1, due to the Ca:P ratio of bone. However, several studies suggest that the Ca:P ratio is not critical unless the ratio is greater than 7:1 or less than 1:1 (Wise, Ordoveza & Barrick, 1963; Call et al., 1978; McDowell, 1992). When animals are fed P deficient diets, high levels of Ca may reduce the absorption of P (Schneider et al., 1985), due to a reduction in rumen P solubility and also a reduction in dietary P availability at sites further down the gut (Field, Kamphues & Woolliams, 1983). Therefore, it seems that ruminants can tolerate a wide range of Ca:P ratios as long as the dietary supply of each mineral is adequate and their vitamin D status is adequate. Field and Woolliams (1984) found substantial variation in the coefficient of absorption of P between, but not within, sets of monozygous twin sheep. Thus, variation between animals in their capacity to absorb P appears to be genetically related (Field, Kamphues & Woolliams, 1983).

### *Faecal excretion*

Practically all P excretion occurs in faeces, and normally urinary excretion of P is of little significance (ARC, 1980; Betteridge, Andrewes & Sedcole, 1986). However, when dietary P is in excess of requirements, urinary P excretion increases (Morse et al., 1992). Faecal excretion of P may be partitioned into three fractions, namely a) P of dietary origin unavailable for absorption or not absorbed, b) P of endogenous origin that inevitably has to be excreted under actual nutritional and physiological conditions, and c) P of endogenous origin which is excreted to maintain homeostasis (Spiekers et al., 1993; NRC, 2001). From investigations using sheep and goats, it must be concluded that inevitable faecal losses of P are a function of dry matter intake (DMI) rather than of live weight, as earlier thought (Doyle, Egan & Thalen, 1982; Field, Woolliams & Dingwall, 1985). The effects of DMI, of inevitable faecal losses of P, may partly be explained by the large contribution of salivary P to total endogenous P secretion. The total endogenous faecal P may constitute more than 2/3 of the total faecal P in cattle and sheep (Scott et al., 1985; Ternouth, 1989; Coates & Ternouth, 1992; Scott et al., 1995) and the main sources of faecal P in ruminant animals are illustrated in Figure 2.

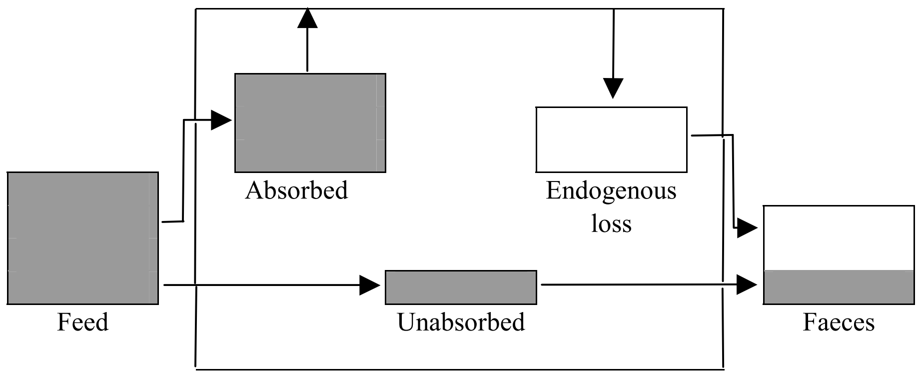


Figure 2. *Block diagram illustrating the difference between apparent and true digestion of feed P (modified from Playne, 1976).*

"Apparent digestibility", measured by the difference between the amounts of P in the feed and faeces, give no information about the different faecal fractions. The "true digestibility", which is arrived at by correcting for the faecal endogenous loss, is commonly referred to as "availability". However, in most experiments no differentiation has been made between the origins of the faecal P, whether exogenous or endogenous P. The P concentrations in products can be measured rather easily, however, the determination of inevitable losses is more critical, and there are no easy and accurate methods available. The amount of endogenous P recycled via saliva must be taken into account and is most appropriately estimated experimentally by quantifying recycling with a tracer (e.g., $P^{32}$). In addition, P must be fed in an amount less than the animal's true requirement to insure the maximum efficiency of absorption. Most studies do not satisfy these experimental specifications. Thus, the true absorption coefficient (TAC) is generally unknown and the value given is an underestimation of true absorption (NRC, 2001). It must be kept in mind that the apparent digestibility of P is lower, mainly because of the large endogenous faecal excretion, and not equivalent to the TAC. However, apparent digestibility still provides a lot of information if it is accurately measured. Even though the apparent digestibility of P is commonly used as a measurement of P digestibility, there are marked differences between studies in the time spent measuring the apparent digestibility (Brintrup et al., 1993; Spiekers et al., 1993; Valk, Šebek & Beynen, 2002). The duration in collecting faeces differs from taking spot samples (Wu, Satter & Sojo, 2000) to total collections for several days (Morse et al., 1992), and there is a lack of information about the variations in P excretion over time.

### *Bone metabolism*

The P content of bone ash is 16-17% or about 4-4.5% of wet bone tissue. P is present in bone, along with calcium (Ca), principally as hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$], and as calcium phosphate [$Ca_3(PO_4)_2$]. The mineral metabolism of bone involves not only the deposition of Ca and P during growth but also the processes of storage and mobilization, which occur throughout life. As distinguished from the shaft, the spongy bone (trabeculae) are lacelike structures and are the principal site in which a reserve of Ca and P is deposited for mobilization to meet the needs, when necessary. The trabeculae are located close to the epiphyseal ends of the bones, where the blood supply is greatest. They provide the Ca mobilized by the parathyroid hormone (PTH) to maintain the level in the blood. During heavy lactation, the reserves are drawn upon to meet a part of the requirements for the minerals secreted in the milk and they also may be drawn upon during pregnancy. This depletion of the reserves generally involves no physiological harm, as long as they can be restored with an adequate diet during periods when the body has reduced needs for Ca and P, e.g. during the dry period for the dairy cow (Maynard & Loosli, 1979). The bones of a 500-kg cow contain about 4 kg of P, some of which can be withdrawn and returned to the blood during osteoclastic resorption of bone.

The hormonal control of P homeostasis appears to be secondary to that of Ca, and it is arguable whether there is any specific and effective hormonal regulation of P metabolism. In Ca metabolism, one of the major regulators is PTH, which also has some effects on P metabolism (Horst, 1986). When the level of Ca in the blood is low the PTH level increases, which induces the kidney to produce more 1,25-dihydroxycholecalciferol. This in turn enhances the intestinal absorption of Ca and

P. PTH also stimulates the activity of the osteoclasts and thereby the mobilisation of Ca and P from bone. For every ten ions of Ca that are mobilized from bone, six phosphate ions also are released into blood circulation and contribute to an increase in the pool of P in the blood. The hormone calcitonin has effects opposing that of PTH, and stimulates bone deposition of the minerals, depresses intestinal absorption and stimulate salivary P secretion.

## Sources of P

P is present in variable amounts in almost all common feedstuffs. The P levels are dependent on the plant species, the level of soil fertility and the stage of maturity at the time of consumption. P concentrations decline as forages mature. Consequently, there are large differences in their P content, although the seeds are uniformly higher in P content than the roughages. Cotton seed meal, oilseed meals and wheat bran are feedstuffs high in P content, while sugar beet pulp is an example of a feed which is very low in P content. About 35-70% of P in plants is in the form of phytate P, which is mostly unavailable for monogastrics, but rumen microbes are able to digest phytic acid so that much of the phytate-bound P is available for absorption in ruminants (Morse, Head & Wilcox, 1992; Herbein et al., 1996). However, factors such as processing techniques, dietary protein content, starch degradability and grain content of the diet may affect the availability of feed P (Braithwaite, 1976; Konishi et al., 1999; Guyton et al., 2000). There are difficulties in setting absorption coefficients of P for all feedstuffs, as most studies are performed in different ways, with differences in dietary P-level, DMI, NDF-content and milk yield. The true absorption coefficients are generally unknown, and most studies include measurements of apparent absorption, sometimes with estimations of endogenous P and only to a minor extent in sacco studies, in vitro studies or tracer technique. Summarising data from the literature, Minson (1990) concluded that the apparent absorption of P from legumes is higher than from grasses.

Most animal diets require supplemental sources of P in addition to that present in the common feedstuffs. There are a number of supplemental sources and the major types include calcium phosphates, ammonium phosphates and sodium phosphates. Monosodium phosphate has a P content of about 25%, which is the highest P content of all feed phosphates, while soft rock phosphate has the lowest P content, about 9%. However, di- and monocalcium phosphate are the most common feed phosphate products sold. Most supplemental P sources are derived from natural rock phosphates, but in their natural form they are unsuitable for direct use. Rock phosphates must be chemically treated so that the P they contain is changed into the digestible orthophosphate form ($PO_4^{3-}$), and furthermore, unsuitable impurities such as fluorine, cadmium, arsenic, mercury and lead, must be removed. There are wide differences in the estimated absorption coefficients of P from various supplemental mineral sources, differing from 90% for monosodium phosphate, 75% for dicalcium phosphate, to 30% for soft rock phosphate (NRC, 2001). Furthermore, in turkeys it has been shown that there are significant differences in the available P content between different types of phosphate, as well as between the same phosphates from different sources (Waibel et al., 1984).

## Dietary requirements

The dietary requirement of P is the sum of the requirements for absorbed P for maintenance, growth, pregnancy, and lactation, divided by the true absorption coefficient (TAC) for P from the diet. The maintenance requirement of P is the endogenous faecal loss (inevitable faecal loss) when P supply is just below or just meets the true requirement. The growth requirement of P is the sum of the amount of absorbed P accreted in soft tissues, plus that deposited in skeletal tissue. The pregnancy requirement of P is the amount of absorbed P accreted in conceptuses (foetus, foetal fluids and membranes, placentomes and uterine tissues); and the requirement for absorbed P for lactation is equal to daily milk yield multiplied by the percentage of P in milk. The factorial method is based on the technique of using radioisotopes to distinguish between the endogenous and exogenous faecal P excretion and to measure the TAC. It should be noted that most experiments of this type were performed using sheep and goats, rather than cows. The TAC in the denominator of the factorial equation potentially has more influence on the final computed dietary requirement than any of the single or combined requirement values for absorbed P. The smaller the TAC, the greater will be the calculated dietary requirement (NRC, 2001). However, the TAC differs between countries, from 55% in Sweden (Spörndly, 1999) to 70% in Germany (GfE, 1993), depending on which working group has established the value. AFRC (1991) have different values of TAC, being 64% and 70% for forages and concentrates, respectively. Recently, NRC (2001) changed the TAC of P, from 50% for all feeds to 64% for forages, 70% for concentrates and 30-90% for various inorganic supplemental mineral sources of P. The values for maintenance, growth, pregnancy and lactation also differ between countries.

The P requirements can be expressed in several ways: in amounts per day or per unit of product, such as milk or weight gain, or in proportions, such as percentage or mg/kg of the whole diet. The required amounts are more precise, but not invariably independent of variations in total food intake (AFRC, 1991). Proportions have the merit of simplicity and have obvious practical advantages, so long as the total diet is palatable (Underwood & Suttle, 1999). In most European countries the maintenance requirement is based on liveweight, whereas in the UK and Germany, as well as the USA, it is based on DMI. The AFRC (1991) hypothesized that the inevitable faecal loss of P is determined mainly by DMI, and not by liveweight. This was based on data showing that if DMI is increased, salivary flow rate and total salivary P secretion increase despite a fall in salivary P concentration, thus indicating that inevitable faecal loss of P are dependent upon DMI. As mentioned earlier, there is a lack of data based on dairy cows. However, Spiekers et al. (1993) used dairy cows and recommend feeding 1.2 g P per kg DMI as the factor for inevitable faecal P losses in dairy cows when calculating net requirements of P, assuming a P availability of 100%. This value was adopted by the NRC (2001), by assuming a TAC of 80% at maintenance, they set the maintenance requirement at 1.0 g/kg of DMI. The AFRC (1991) have set the maintenance requirement of P to ($0.693 \times$DMI (kg/day) -0.06) and if the diet contains ≥50% roughage, the calculated value should be increased by a factor of

1.6. Consequently, some confusion may be arising from the use of different criteria and assumptions by national authorities when calculating P requirements.

## Deficiency and toxicity

Extensive areas of P deficient soils occur throughout the world, especially in tropical and subtropical areas, and a deficiency of this element can be regarded as the most widespread and economically important of all the mineral disabilities affecting grazing livestock. Besides bone-chewing, severe P deficiency may lead to weight loss, rough haircoat, abnormal stance, lameness, spontaneous fractures in the vertebrae, pelvis and ribs, inability to properly heal fractures, markedly demineralised bones, osteoporosis, osteomalacia, weakness and death (Shupe et al., 1988). Marginal deficiencies have been reported to result in foot lesions and recumbency in high producing early lactation cows (Gerloff & Swenson, 1996).

Supplemental P, given in large oral doses, are not considered highly toxic as dairy cattle are quite adept at excreting excess absorbed P, mainly via faecal excretion (Challa, Braithwaite & Dhanoa, 1989). However, overfeeding of P has been reported to reduce the apparent absorption of magnesium (Schonewille, Van't Klooster & Beynen, 1994) and increase the incidences of milk fever and hypocalcemia at parturition (Reinhardt & Conrad, 1980). Long term feeding of excess P can cause problems to the Ca metabolism, inducing excessive bone resorption (NRC, 1980). A relative excess of P in relation to Ca can result in urinary calculi, which is the formation of stones or calculi in the kidney or bladder with resultant obstruction of urine excretion (Blood & Henderson, 1968).

## The environmental situation in Sweden

Several countries have, during the last decade, revised their P recommendations for cattle, and the main reason for this is environmental concern (GfE, 1993; NRC, 2001; Valk & Beynen, 2003). In the USA, P losses from livestock farms account for as much as 47% of the P loading to bodies of surface waters, depending on the watershed (Smith & Alexander, 2000). In Loch Neagh, the largest fresh water lake in the British Isles, agriculture contributes 62% of the P input (Watson, 2000). So, how is the environmental situation in Sweden? Around 1970, most of Swedens' municipal wastewater treatment plants installed chemical or advanced treatment, which can eliminate 90% or more of the P content of unprocessed wastewater. Since the mid-1970s, practically all Swedish urban households and light industries have been connected to municipal wastewater treatment, and about 95% of emissions today undergo both chemical and biological treatment. This means that Sweden, together with Finland, has the world's most comprehensive system of wastewater treatment. However, eutrophication has remained a serious problem in many Swedish inland and coastal waters and the main reason is nutrient emissions from agriculture. Between the 1920s and the 1970s, P fertilisation of arable land doubled, with increasing quantities of P seeping into nearby waters. Today, P fertilisation has again been brought down to the 1920s level, but the amount of P stored in arable land remains undiminished. It is the intensively farmed plainlands of Central and Southern Sweden that today have the most eutrophic lakes and watercourses. The waters here have always been more eutrophic than in the forest regions further north, but farming has done a great deal to accentuate their eutrophication. P concentrations in the plainland rivers today are rated about five times the original levels. Altogether, more than 14,000 of Sweden's 90,000 lakes have such high P content (25 µg/l or more) that they can be termed eutrophic. In 1997, The Swedish Parliament established 15 environmental quality objectives, such as "Zero Eutrophication" and "Good-quality groundwater", to guide Sweden towards a sustainable society (Swedish Environmental Protection Agency, 2003). Consequently, P excretion and P nutrition in dairy cows is of great concern in Sweden, and there is a large interest in the prospect of revised and, if possible, lower the P recommendations.

# Aims of the thesis

The overall aim of this thesis was to increase knowledge about the digestibility and requirements of P in dairy cows.

The specific aims were to study:

- The effects of varying the amounts of dietary P on the apparent digestibility and excretion of P.
- Variations in daily P excretion in faeces.
- Differences in the apparent digestibility of P from varying P sources.
- The applicability of biochemical bone metabolism markers.
- If it is possible to improve P utilisation by taking advantage of the naturally occurring bone resorption in early lactation.

# Materials and methods

This thesis is based on three different studies, carried out at Kungsängen Research Centre, Swedish University of Agricultural Sciences, SLU, in Uppsala, 1998-2002. Uppsala Animal Ethics Committee approved all experimental procedures, which are described in detail in each paper. Methodological aspects of general interest are discussed here.

## Experimental designs and animals

The apparent digestibility of P was measured in all studies. In the first study, referred to as the Level-study (paper **I**), five cows were assigned to a Latin Square Design of 5×5 with periods of three weeks. In the second study, referred to as the Source-study (paper **II**), four cows were assigned to a Latin Square Design of 4×4 with periods of four weeks. In the third study, referred to as the Lactation-study (papers **III** and **IV**), two groups with 10 and 11 cows were randomised on two different treatments, covering two dry periods and the lactation between these dry periods (Table 1). Analysis of variance was performed on all data using the mixed model procedure (Littell et al., 1996) in SAS® System for Windows release 6.12 (paper **I** and **II**) and release 8.01 (paper **IV**), and Minitab Statistical software release 13.31 (paper **III**). Models are described in each paper. Multiparous cows of the Swedish Red and White Breed from the experimental herd at Kungsängen Research Centre were used.

In all studies, total collections of faeces were conducted manually for 24 hours per day for 5 consecutive days during each collection period (Figure 3). Faeces were collected in rubber tubs, weighed for each 24-h period and mixed with 10% water prior to being homogenized. Representative aliquote samples were taken and frozen on a daily basis. Subsequently, samples were thawed and combined on a weekly basis. After freeze-drying, P was degraded in nitric acid and total P was then determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES). During the collection periods, the bedding was removed. Milk yield and milk composition were recorded monthly in the Level-study, and weekly in the Source- and Lactation-studies. Spot samples of urine were taken in all studies.

Figure 3. *Total collection of faeces.*

In the Lactation-study blood samples were taken every, to every third week, during the entire study. The samples were obtained from the coccygeal vessel and collected into evacuated tubes containing sodium heparin as an anti-coagulant (Venoject, Terumo, Leuven, Belgium). The samples were taken at approximately the same time of the day (between 10.00 h. and 11.00 h.) and kept on ice until centrifuged (10 min at 1800×G) within one hour of sampling. The plasma samples were stored at -80°C until analysis. Samples were analysed for osteocalcin (Intact Human Osteocalcin EIA kit, Biomedical Technologies Inc., Stoughton, USA; assay validated for human, bovine, dog, goat and pig) and CTx (Serum Cross Laps™ One Step ELISA, Osteometer BioTech A/S, Herlev, Denmark; assay validated for human, bovine, horse, sheep, goat, pig, dog, elephant and chicken) according to respective assay procedures. The Serum CrossLaps® ELISA was used for quantitative assessment of bone resorption. The assay detects C-telopeptide fragments of collagen type I (CTx) generated during osteoclastic bone resorption.

Intact Human Osteocalcin EIA kit is used for quantitative assessment of bone formation in plasma. The assay detects only intact osteocalcin (OC), which is synthesized de novo by the osteoblasts, and it eliminates any potential confounding interference by circulating fragments.

## Feeding

The cows were kept in tie-stalls and fed individually. They had free access to water and a lick stone of pure NaCl, and feed was offered in equal amounts, 5 times per day. The cows were fed restricted diets, calculated according to recommendations (Spörndly, 1999) and kept indoors throughout the studies. The grass silage consisted of approximately 60% timothy grass, 30% meadow fescue and 10% red clover, and the approximate main ingredients in the concentrates are shown in Table 2. Inorganic P was added in the form of monosodium phosphate. Grass silage and concentrate were fed in separate troughs and feed refusals were weighed separately.

Table 2. *Main composition (g/kg) of concentrates used in the studies*

| | Level-study | Source-study | | | | Lactation-study |
|---|---|---|---|---|---|---|
| Concentrate | P40 - P140 | MSP | WMB | SSP | RS | LP and NP |
| Oats | 450 | - | - | - | 50 | 290 |
| Wheat | - | 180 | 440 | 150 | 150 | 160 |
| Rape seed products | - | - | - | - | 270 | 80 |
| Soya bean meal | - | - | - | - | - | 80 |
| Sun flower/palm kernel | - | - | - | 310 | - | - |
| Sugar beet pulp | 430 | 580 | 430 | 370 | 410 | 280 |
| Alfalfa meal | - | 90 | - | 70 | 60 | - |
| Corn gluten meal | 75 | 60 | 65 | 50 | 40 | 50 |
| Potato protein | - | 30 | 35 | 15 | 15 | 20 |
| Vegetable oils | 25 | 30 | 20 | 30 | - | 25 |
| Urea | 10 | 13 | - | - | - | - |

## Paper I: Different levels of P intake (the Level-study)

Different quantities of monosodium phosphate (MSP) were added to a basal diet to obtain daily P-intake levels of 44 (P40), 67 (P65), 92 (P90), 117 (P115) and 142 g (P140), which corresponds to 0.24, 0.37, 0.51, 0.64 and 0.78% P of DM, respectively. The basal diet consisted of 7.8 kg DM of grass silage, 1.0 kg DM of hay and 8.9 kg DM of a pelleted concentrate with a low P content. The average milk production was 25 kg energy corrected milk (ECM) per day at the onset of the trial and the cows were in gestation month 3-4 when the trial started.

## Paper II: Different P sources (the Source-study)

Four different concentrates with monosodium phosphate (MSP), rapeseed (RS), sunflower seed/palm kernel (SSP), or wheat middlings/bran (WMB) as the main P-source (56-67% of P in the total diet) were offered, together with grass silage. The rumen solubility of P in the four concentrates was studied in vitro. Cows were in lactation weeks 12-14 at the start of the trial, milking an average of 36 kg ECM.

### Paper III: Biochemical bone markers (the Lactation-study)

The practicability of OC and CTx as biochemical bone markers was evaluated. This paper is a part of the Lactation-study, including the eleven cows that participated during the entire study.

### Paper IV: P intake and stage of lactation (the Lactation-study)

Two different levels of P, 0.32% (LP) and 0.43% (NP) of DM, were fed during the first four months of the lactation followed by one level, 0.43% of DM, from the fifth month until the end of the lactation. During the first and second month of the dry periods, dietary P levels were 0.31% and 0.34% of DM, respectively. The apparent digestibility of P was measured during 5 different periods (C1-C5), spread over the entire study. The cows were blocked according to calving date and, thereafter, allotted to the two treatments. Cows that were not pregnant after two inseminations were excluded from the trial after about 30 weeks in lactation.

# Results and comments

## The Level-study (paper I)

The cows showed marked differences in faecal P output between days within each collection period. The coefficients of variation were in the ranges 5-14% and 2-15% for the lowest and highest P intake, respectively. The apparent digestibility of P for P40, P65, P90, P115 and P140 was calculated to 37.0, 28.5, 22.0, 20.9 and 21.7%, respectively. Values are least square means (LSM). The apparent digestibility of P40 was significantly higher than apparent digestibility of P90, P115 and P140 ($p \leq 0.006$). In periods 1 through to 5, the apparent digestibility of P were 26.4, 30.0, 31.7, 24.6 and 17.6%, respectively (LSM values). The apparent digestibility in period 5 was significantly lower than in period 2 ($p=0.03$) and 3 ($p=0.01$). The mean concentrations of P in urine were 16, 16, 41 and 76 mg/l for P40, P90, P115 and P140, respectively. Milk production decreased markedly during the experiment and was estimated to 23.0, 20.1, 17.3, 13.4 and 5.8 kg per day for periods 1 through to 5, respectively.

*Comments on paper I*

The differences in daily faecal P excretion were largely due to differences in the daily amounts of faecal DM. Due to these large differences, there is a need for sampling to take place for several consecutive days in P metabolism studies. There was a linear relationship between the amounts of P consumed and excreted in faeces ($r^2$=0.96). The slope of the regression indicates that for each gram of increased P intake, 0.86 g would be excreted in faeces. This high value is most likely caused by the high P intake in relation to the requirements and also due to the relatively low milk yield. It clearly demonstrates how pointless the feeding of P in excess of requirements actually is.

The apparent digestibility of P decreased with increased P intake. The overall values of apparent digestibility may seem low, but as excess P is excreted via faeces the low apparent P digestibility simply reflects that P intake was clearly in excess of the cows' requirements. The decrease in apparent digestibility in period 5 was probably due to the reduction in milk production. As the recording of milk production did not precisely correspond to the collection periods, the milk production is estimated, and consequently even P retention is an estimate. Towards the end of lactation the estimated P retention increased markedly. This increase may imply that the cows, at this time, have begun to replenish bone P in preparation for the P mobilization which takes place when the next lactation period begins.

## The Source-study (paper II)

There were no significant differences between treatments in the apparent digestibility of P, which were 49.8, 52.2, 47.4 and 51.9%, and milk production, which were 36.3, 35.9, 33.2 and 37.2 kg ECM, for MSP, WMB, SSP and RS, respectively. However, there were significant differences in the total P intake, which varied between 82.4 and 93.0 g per day. The in vitro digestibility of P in rumen fluid was calculated to be 94.3, 89.5, 85.2 and 74.3% for MSP, WMB, SSP and RS, respectively.

### *Comments on paper II*

The contribution of P from the individual P sources being investigated was 56-67% of total P in the diet, which should be considered as high enough to make comparisons possible. The P sources included in this experiment were of both inorganic and organic origin, and also showed differences in in vitro digestibility. Therefore, differences in digestibility could be expected. However, there were no differences in apparent digestibility between the P sources. There is still a possibility that there were differences in the true absorption, but only to a minor extent and thereby not possible to measure only by calculating the apparent digestibility. The results indicate that under normal feeding conditions for dairy cows, the need for using different absorption coefficients for the different concentrate ingredients used in this experiment is questionable.

## The Lactation-study (paper III and IV)

There were no significant differences in milk production between treatments during the different periods, and no differences in average daily milk production during the whole lactation, which were 30.5 and 30.4 kg ECM for NP and LP, respectively. There were no significant differences in the plasma concentration of

CTx between treatments in any period, but plasma concentration of OC were significantly higher for LP in the period 17 to 24 weeks of lactation and there was a significant period × treatment interaction for OC ($p<0.05$). During C1 and C2 P intake was significantly higher for NP, as planned, and P in faeces was significantly higher for NP. During these periods, the apparent digestibility of P was significantly lower for NP, but there were no differences in P retention or kg of ECM. P retention increased during C4 and C5 and there were significant effects of period ($p<0.05$). During C3 to C5 there were no significant differences between treatments whatsoever. Spot samples of urine showed an average P content of 0.013 and 0.012 g/litre for NP and LP, respectively, and spot samples of milk showed an average P content of 0.94 ± 0.11 g/litre.

### *Comments on paper III*

OC is a specific product of the osteoblast and was therefore used as a measurement of bone formation. CTx is a specific product of the osteoclast and was therefore used as a measurement of bone resorption. The results showed a large variation in the dynamics of bone metabolism during lactation that was not related to milk production. The plasma concentration of CTx showed the highest value the first week after parturition. It then declined successively and reached the bottom level about 33 weeks after parturition. This low level was then maintained until parturition. OC showed a different pattern. The lowest values were registered around parturition. The concentration rose after parturition and reached a plateau in mid lactation, followed by a continuing reduction until next parturition.

### *Comments on paper IV*

Cows fed the LP-diet excreted significantly lower amounts of P in faeces and had a higher apparent digestibility of P than cows fed the NP-diet. Still, the P retention was positive with no significant differences between the treatments. If the LP-diet would not provide sufficient P, in relation to the requirements, the apparent digestibility and P retention would have increased when the LP-group changed to the NP-diet. The fact that there were no changes in the apparent digestibility and P retention when the LP-group was started on the NP-diet, underlines that the LP-diet provided sufficient P in early lactation. The P retention increased in both groups during the end of lactation and the dry period, which is likely to reflect the requirements for growth of the foetus as well as the replacement of bone P in preparation for the next lactation. During the dry period the apparent digestibility of P was only around 28%, and it can therefore be assumed that P was fed in excess during this time.

There was a considerable difference in CTx between dry period 1 and dry period 2. It is likely that this is related to the fact that 77% of the cows were in their first lactation when dry period 1 started, still growing and thereby having a higher bone turnover. As the bone resorption was very low during the end of the lactation and during the dry period, while the bone formation was relatively high, the total bone formation must have been highest during the end of lactation and the beginning of the dry period. This indicates that an adequate intake of Ca and P probably is of greatest importance during this period.

# General discussion

## Experimental designs in P studies

The most common approach for P digestibility studies has been to measure the apparent digestibility of P. This approach has been used extensively, but there does not seem to be an agreement in the experimental design. There is a large variation between studies in the duration of the faeces collection, from taking spot samples to total collection for nine consecutive days (Brintrup et al., 1993; Spiekers et al., 1993). There are two factors that could explain this variation. Total collection of faeces is a very labour intensive method and there are no literature sources that have determined the necessary duration of these collections. Wu et al. (2000) found a considerable diurnal variation in faecal P concentrations, showing that spot sampling faeces is an unreliable method for the determination of P excretion. The study reported in paper **I**, addressed the issue of the time duration needed in order to obtain valid data for P excretion from total collections of faeces. The results clearly show that it is important to collect faeces for several consecutive days. In order to determine how precision in the measures increases as the duration of total collection increases, the residual of the covariance was analysed for one to five days. However, this analysis did not show that collection for less than five days would be sufficient and therefore, total collection has to continue for at least five consecutive days in order to obtain valid data on P excretion.

In addition to measures of P excretion in faeces, most P digestibility studies report P content in urine. As is the case with faecal P, the sampling techniques for urinary P determinations vary between studies. In some studies spot samples are taken (Spiekers et al., 1993; Wu, Satter & Sojo, 2000) while others perform total collection of urine (Morse et al., 1992; Valk, Šebek & Beynen, 2002). Several authors agree that P excreted in urine is negligible under normal P intake (Spiekers et al., 1993; Wu et al., 2000; Valk et al., 2002). However, when P is fed in great excess, significant amounts of P may be excreted in the urine (Morse et al., 1992; paper **I**, **II**). In paper **IV**, when P was fed close to the requirements, urinary P concentration was on average 12 mg/litre. In paper **I**, when P intake was about 3 times the requirements, urinary P concentration was on average 76 mg/litre, which is more than 6 times higher than when P intake corresponded with the requirements. The lower amount must be considered as negligible, and based on these data it can be concluded that urinary collection is unnecessary when P intake is low or close to the requirements. However, if experimental designs with excess P intake are applied, urine should be collected. There seem to be individuals with homeostatic malfunction or impaired physiological function that affect urinary P excretion. In the Lactation-study, one individual cow excreted up to 70 times more P in urine than the other cows did. Yet, no health problems were noticed and blood samples revealed no malfunction of the kidneys. Therefore, before starting a P digestibility study, it may be wise to take spot samples of urine in order to detect, and avoid, animals with abnormal urinary P excretion.

According to Adams (1975) and Kertz (1998) there is considerable variation in P content within the same kind of concentrates and forages fed to dairy cows and this is the most likely explanation of the differences in P content between the concentrates in the Source-study. In the Lactation-study, for practical reasons, batches of 5 tons of concentrates had to be prepared. The recipes were re-optimised for each batch, according to the current P content of the feed ingredients. There were generally only minor variations in the P content between batches, but one batch had to be excluded from the experiment due to a divergent P content. The largest variation in P content was in potato protein (2.1-4.6 g/kg DM) and wheat (2.9-4.8 g/kg DM). Due to the variation in P content within feed ingredients, it is important to analyse P content continuously, for precise and accurate diet formulation.

The variation in the P content of milk is reported to be between 0.83 to 0.85 g/kg (Wu, Satter & Sojo, 2000), 0.95 g/kg (Paul & Southgate, 1978) and 0.90 to 1.00 g/kg (Flynn and Power, 1985). In the Source- and Lactation-studies, averages of 0.93 g and 0.94 g P per kg milk were found. This is very close to the value of 0.90 g P/kg milk, which is used in several models to compute requirements for absorbed P (INRA, 1988; AFRC, 1991; GfE, 1993; NRC, 2001). Spot samples of milk appear to be sufficient to obtain valid data on the P content of milk and according to Holt (1985) there is little variation in the P content throughout lactation.

## Absorption and faecal excretion of P

In paper **I**, it was clearly shown how the apparent digestibility of P decreased with increased P intake. For each gram of increased P intake, 0.86 g was excreted in faeces. In the study of Morse et al. (1992), 0.55 g P was excreted in faeces, for each gram of increased P intake. One explanation for this difference could be the higher milk yield in the study of Morse et al. (1992). Valk, Šebek & Beynen (2002) showed that a higher proportion of the ingested P is transferred to milk, and less is excreted with faeces, when milk yield increases. This leads to an increased percentage of apparent P digestibility. It is difficult to compare studies, due to differences in experimental design, but current literature suggests that there are diverse factors influencing P utilisation. According to Schneider, Boston & Leaver (1982), the fall in absorption efficiency at high intakes of P could be part of a homeostatic mechanism, controlled by so far unidentified hormonal systems or be a result of a progressive saturation of the absorption mechanism. Care, Barlet & Abdel-Hafeez (1980) showed in sheep that the net rate of P absorption increased with increasing luminal P concentration in the jejunum, up to a concentration of approximately 7 mmol/litre when the absorption system became saturated. They suggested that this relationship would be compatible with the saturation of a putative carrier mechanism during an absorptive process involving facilitated diffusion. Scott et al. (1985) showed, in sheep, that increasing P intake led to an increase in net intestinal P absorption, a rise in plasma P concentration, an increase in salivary P secretion and an increase in faecal endogenous P excretion. Overall net intestinal absorptive efficiency for P did, however, decrease with increasing P intake, so that changes in faecal endogenous P excretion were in part due to increased salivary P secretion and in part due to a reduction in overall absorptive efficiency. As the homeostatic regulatory system presumably is similar in cows and sheep, it is likely that there is a level of P intake where P absorption is saturated in cows as well. In the Level-study, we could not measure the degree of actual P absorption. The increase in urinary P excretion at the higher levels of P intake, reflected the increase in P absorption. However, it seems very likely that the absorption system to some extent became saturated at the higher P intake levels, as otherwise enormous amounts of P must have been recirculated via the saliva in those cows. Schröder, Rübelt & Breves (1993) suggested that about 65% of active jejunal P transport is mediated by a $Na^+$-dependent transport mechanism in sheep. However, Shirazy-Beechey et al. (1991) showed a $Na^+$-independent but $H^+$-driven P transport mechanism, also in sheep. Results obtained from cattle under normal physiological circumstances are scarce, but, we know that P absorption, salivary P, bone resorption, and excretion of urinary P and endogenous and exogenous faecal P all play a part in the homeostatic control of P (Vitti et al., 2000). It is important to keep in mind that the cow has no ability to store P above the requirements. If P is not immediately useful for the cow, then P will be excreted in the faeces as part of the endogenous losses.

## Effects of P source

The results in paper **II** suggest that there are no differences in the apparent digestibility of P between the concentrates investigated. This is supported by Knowlton et al. (2001). However, data obtained by Khorasini et al. (1997) showed that the forage source can influence the extent of P absorption. Cows fed triticale silage secreted more P into the rumen than cows fed alfalfa and barley silages, probably because of the higher NDF concentration in the triticale silage, resulting in increased saliva production (Khorasani et al., 1993). Still, there were no differences in the apparent digestibility of P between diets (Khorasini et al., 1997). This indicates that to calculate the apparent digestibility of P alone is not an accurate method when comparing differences in P digestibility between feedstuffs. In the Source-study, the concentrates were also studied in vitro by incubation in rumen fluid. The RS-diet showed the lowest P solubility compared to the other diets. Bravo et al. (2000) also found rape seed meal to have a lower P solubility compared to other concentrates, using in sacco studies. However, we do not know how much P that eventually will become soluble in the acid region of the small intestine. Sehested & Weisbjerg (2002) studied the release of P from 21 feedstuffs using the nylon bag technique. The ruminal availability varied significantly between feedstuffs. However, the total release of P from concentrates, i.e. mobile bags passing through both rumen and intestine, was 94-99%, indicating a very high total P solubility. There is a possibility, however not likely, that some of the released P was bound as phytate. Although differences have been noted in the rate of hydrolysis of phytate-P between concentrates in vitro, when the faeces of dairy cows fed rations containing 0.17% and 0.38% P as phytate-P were analysed, only trace amounts of phytate-P were observed (Morse, Head & Wilcox, 1992). There is research indicating the need for different TAC for forages and concentrates (AFRC, 1991; NRC, 2001). The results in this thesis do not show a need for introducing P recommendations with different TAC for different concentrate ingredients.

## The use of biochemical bone markers

In addition to studies of P balance, it is of great interest to study P status in the cow. One method used in P status studies is to determine P in bone tissue on rib samples that are removed surgically. The specific gravity of bone can then be measured and bone composition is expressed on a volume basis, relating to bone mineral storage (Wu et al., 2001). Another method that is described in the literature is to perform radiography on the tail (Shupe et al., 1988). Both of these methods are quite limited. They are suited for studying P depletion only and will not give information about the continuing skeletal metabolism. Removing a part of the rib bone, or even slaughtering the animals (Blair-West et al., 1989), are invasive methods and offer little opportunity of repeated measures within an individual animal and because of this they require large groups of animals. Biochemical bone markers offer a way to study bone metabolism and P status from blood samples, which allows repeated samples to be taken from the same individual. This method is used frequently for studies of osteoporosis and bone resorption in humans (Sowers et al., 1995; Uemura et al., 2002). Bone markers have only been used in a few cattle studies so far and mainly around parturition (Lappeteläinen et al., 1993; Liesegang et al., 2000). Two markers of bone formation are the extracellular matrix protein osteocalcin (OC) and the enzyme alkaline phosphatase, which are both synthesized by bone forming osteoblasts. Mosel and Corlett (1990) measured these two markers and found alkaline phosphatase to be less valid than OC as a measure of osteoblast activity. Alkaline phosphatase can also be synthesized by other tissues, for example the liver. Therefore, OC provides a more specific measure of osteoblast activity and was chosen as marker of bone formation in the Lactation-study. CTx is generated during osteoclastic bone resorption. It has mainly been used to monitor human bone resorption changes, as an aid in the study of postmenopausal osteoporosis and the assessment of bone resorption in human patients. OC and also CTx proved to be very indicative of bone formation and resorption during lactation. However, it is important to remember that the bone markers do not show the actual P content of bone, but the bone formation and resorption, respectively. In the Lactation-study, the bone formation was highest during mid- and late lactation, and bone resorption was highest in early lactation. It has been suggested that bone resorption and formation are coupled and in balance, i.e., when resorption is increased, bone formation is also enhanced (Eriksen, 1986). However, the results from the Lactation-study showed that bone resorption and formation are not coupled throughout lactation. This is in agreement with Liesegang et al. (2000), who showed that bone resorption and formation was uncoupled after parturition. From the OC to CTx ratio, we can conclude that total bone formation was highest from about lactation week 30 on to parturition (Figure 4). It is therefore likely that P supply is very important during this period.

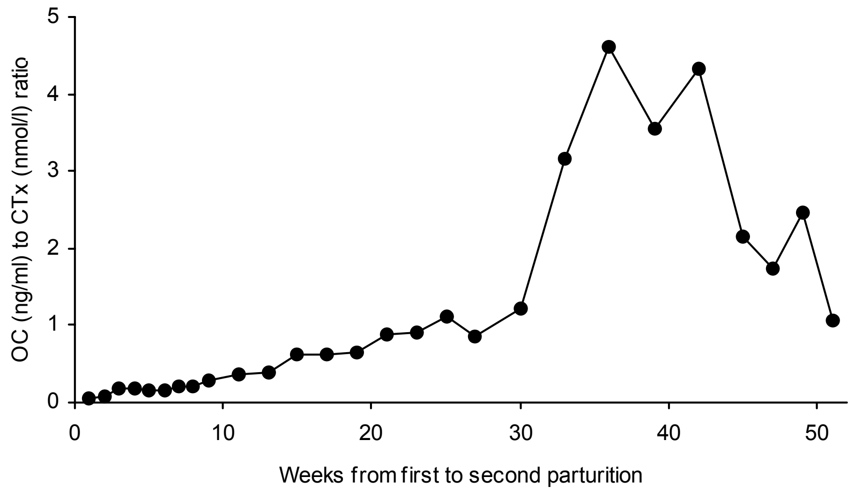


Figure 4. *The OC (ng/ml) to CTx (nmol/l) ratio in eleven cows from their first to second parturition in the Lactation-study. Cows were dried off about eight weeks before parturition.*

Both OC and CTx have been shown to have diurnal rhythms in many non-ruminant species, which may confound the interpretations of results (Heuck, Skjaerbaek & Wolthers, 1998; Liesegang et al., 1999). In the Lactation-study blood was always collected at the same time of the day, in order to avoid any confounding effects of any possible diurnal variation. Also, OC and CTx were measured using hourly samples from 24 hour blood samplings of three cows and no diurnal patterns were observed (Figure 5).

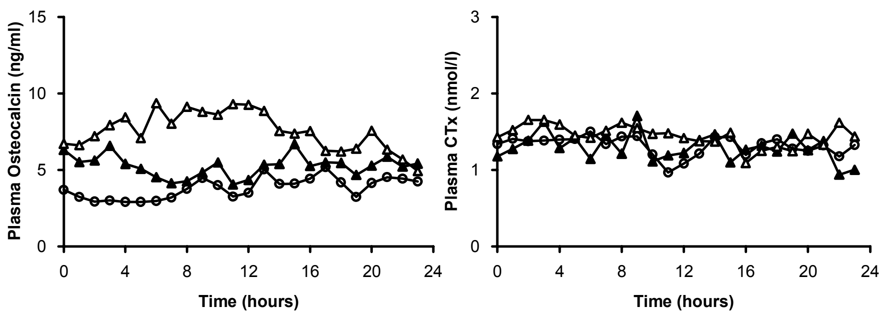


Figure 5. *Diurnal variation in plasma OC and CTx from three cows.*

## Current P recommendations

The exact dietary P requirement is individual, and greatly influenced by all factors that limit its' absorption. It is, therefore, not possible to produce general requirements for P intake that are precise on individual level. Most table-values include safe allowances, including the most adverse individual circumstances and leave no animal at risk from deficiency. Hence, they are only recommendations and do not represent true requirements. The history of the Swedish P recommendations began early in the twentieth century. However, in 1965 the P recommendations from the ARC (1965) were adopted. The TAC was set at 55% and the P recommendations for net maintenance were set at 28 mg/kg BW. In 1984 the net maintenance level was reduced to 20 mg/kg BW, and that is the only revision of the Swedish P recommendations that has been made since 1965 (Everitt & Pehrson, 1984).

There has been a lot of research on P requirements during the last 20 years, which has generated new knowledge about P nutrition and metabolism. Therefore, it is time to scrutinize the Swedish P recommendations. As inevitable faecal loss of P mainly is determined by DMI (AFRC, 1991), it does not seem to be proper to have recommendations based on BW. This is also the approach chosen by the countries that lately have revised their P recommendations (AFRC, 1991; GfE, 1993; NRC, 2001). The most recent revision has been made in The Netherlands (Valk & Beynen, 2003). Their recommendations are based on calculations of saliva production, saliva P content and the efficiency of P re-absorption, derived from literature values and experimental results. They recommend (19+1.43×kg milk) g P/day for a 600-kg cow. The current Swedish recommendations are (21+1.8×kg energy corrected milk) g P/day. The difference may not seem overwhelming, but on a yearly basis this means that a Dutch cow will be fed about 5.1 kg less P than a Swedish cow.

The Dutch P recommendations may seem low, at least in relation to the Swedish recommendations. However, in the Lactation-study, the LP-cows received about 75% of the Swedish P recommendations during early lactation. No significant differences in DMI, milk yield, P retention or biochemical bone markers were found during this time, compared to the NP-group, and the P intake in the LP-cows was about 8% lower than the Dutch recommendations. Most P recommendations for lactation are based on kg of milk, while the Swedish system is based on kg of energy corrected milk (ECM), comprising corrections for both fat- and protein-content (Sjaunja et al., 1990). This could be of concern, as about 60% of the P in milk is in the form of fat and protein, while 40% is not (Jenness, 1974; Holt, 1985). Abbas (1999) showed that an increase in milk protein content was associated with a substantial increase in milk P content as well. Furthermore, as feeding plans generally are based on kg ECM, it is practical to also base P recommendations on kg ECM. The net P recommendation for milk production is kg milk×0.9 in the other recommendations mentioned above, but the differences in TAC lead to differences in the recommendations. We know that the true absorption is markedly higher than the apparent absorption. Therefore, based on the results in this thesis, and the research behind several recommendations where the TAC is set to 64-80%

(GfE, 1993; NRC, 2001; Valk & Beynen, 2003), we can conclude that the Swedish TAC of 55% is underestimating P absorption.

During the dry period the cow requires P for maintenance and for pregnancy. According to the results in paper **IV**, the net retention during early and mid lactation was 2.0-4.6 g/day, and the net retention during the dry period was 8.8-9.9 g/day. This indicates that approximately 6 g/day was needed for the growth of the foetus and for bone formation during the dry period. The Swedish P recommendations for pregnancy during the last two months are, on average, 10.5 g/day and consequently the Swedish recommendations during the dry period are likely to overestimate the needs for P during the dry period. This is further supported by the low apparent digestibility of P during the dry period, indicating an excess P intake. According to Annenkov (1982) the net P requirement for foetal growth during the last month of pregnancy is 4.2 g/day, and based on this value the Dutch P recommendation for a pregnant dry cow is 19 g/day (Valk & Beynen, 2003). The Swedish recommendation for a dry cow, during the last month of pregnancy, is 34 g/day.

There are several possible approaches for a revision of the Swedish recommendations. The TAC could be increased in accordance with other recently revised recommendations, new recommendations could be adopted directly from one of the countries that have revised their recommendations recently, or a new approach to P feeding could be developed from the results in the Lactation-study. That experiment showed that it is possible to have a lower dietary P content at the beginning of a lactation, leading to a better total utilization of P. In this system, with a reduced dietary P level in early lactation followed by the present recommended dietary P levels, a large safety margin is included at the end of the lactation and during the dry period. This is the time when the largest part of bone formation takes place. In this way, the dietary P level is optimised according to the bone metabolism.

However, apart from how the revision of the Swedish recommendations will be performed, the TAC has a tremendous impact on the dietary P recommendations of lactating cows. Therefore, more research is a needed to better define the P requirements of the dairy cow and the digestibility of P in feedstuffs. It is also necessary to investigate technologies that may increase the digestibility of dietary P. Most research and cost-share programs for reducing agricultural nutrient loading on water resources have been directed towards agronomic nutrient management and manure handling. However, improving nutrient utilization in the dairy cow with more accurate diet formulation will reduce nutrient losses to the environment and may save farmers money compared with traditional approaches. Perhaps we need to reconsider funding priorities and focus on the amounts of P we put into the system, instead of how to take care of the amounts of P coming out.

# Conclusions

- Increased P intake led to a decrease in the apparent digestibility of P.
- There was a linear relationship between P intake and P excretion in faeces.
- There was a considerable variation in faecal P excretion between days.
- The apparent digestibility of P did not differ between the P sources investigated.
- The biochemical bone markers OC and CTx proved to be indicative of bone formation and resorption.
- There was an appreciable increase in bone resorption immediately after parturition.
- Bone formation was highest during mid lactation.
- It was suggested that the naturally occurring bone resorption in early lactation could be taken into account in diet calculations leading to lower P intake during this time, and consequently less P excretion in faeces.

# Populärvetenskaplig sammanfattning

Ämnet fosfor (P), upptäcktes under 1600-talet av så kallade alkemister i jakten på att framställa guld, och har sedan dess haft stor betydelse inom många olika områden, allt från tillverkning av tändstickor till att utnyttjas som växtnäring. Själva ordet kommer från grekiskan och betyder ”ljusbringande”, då P i fri form kan brinna vid kontakt med syre. P är livsnödvändigt för både människor och djur, och räknas som det mineralämne som har flest skiftande funktioner i kroppen. Mest känt är det för att ingå i skelett och tänder, men finns även i mjölk. I komjölk finns nära 1 gram P per liter. Dagens mjölkkor producerar stora mängder mjölk, och utsöndrar sammanlagt under ett år över 8 kg P via mjölken. Extra P måste därför tillföras fodret, för att kon inte ska drabbas av P-brist. Om kon får för lite P minskar både foderintag och mjölkproduktion, och på sikt blir skelettet skört. Om kon däremot får för mycket P, regleras det genom utsöndring i träcken.

Det är viktigt att vi utfodrar korna med den mängd P de behöver, varken mer eller mindre. Att tillföra ett överskott av P är inte direkt skadligt för kon, men det är inte försvarbart vare sig ekonomiskt eller miljömässigt. Dels är P av hög kvalitet dyrt, och dels måste P i mineralfoder ses som en ändlig naturresurs, eftersom den bryts från berggrunden. Dessutom hamnar överskottet i form av gödsel på våra åkermarker, och om vi tillför marken mer P än den kan hålla kvar, kan det leda till läckage av P till vattendrag och sjöar. En hög P-halt i vattnet kan leda till en ökad algblomning som minskar syremängden i vattnet, vilket i sin tur leder till en förändring i bestånden av olika alg- och planktonarter samt på sikt även en förändring av hela fiskpopulationen. I Sverige arbetar man nu från flera håll med att begränsa P-läckaget från lantbruket, framför allt i de södra delarna där djuruppfödningen är som intensivast.

Övergödning av sjöar har blivit ett allt större problem i många länder, och det finns olika sätt att lösa problemen på. Ett sätt är att utfodra mindre mängder av P, så att mindre mängder kommer ut i träcken. Därför ser man nu på många håll över sina utfodringsrekommendationer av P. De flesta länder har sina egna rekommendationer, baserade på olika forskningsresultat. Rekommendationerna i olika länder bygger dock på samma system, nämligen att en ko behöver P för underhåll, mjölkproduktion samt eventuell dräktighet. Det är svårt att exakt fastställa dessa mängder och det förekommer skillnader mellan enskilda kor. Dessutom tar inte korna upp all P som finns i fodret, det måste därför finnas en säkerhetsmarginal i rekommendationerna. I takt med att nya forskningsresultat kommer, revideras rekommendationerna. Det kan göras på olika sätt. Det vanligaste hittills har varit att justera rekommendationerna för underhåll och/eller mjölkproduktion, eller att ändra den faktor som beskriver hur mycket av P som kon kan utnyttja, och som rekommendationerna baseras på. Den faktorn ligger i Sverige på 55%, och i andra länder varierar faktorn från 50% till 80%. Den stora variationen mellan länder visar tydligt att mer forskning behövs inom ämnet.

Metabolismen av P är speciell hos idisslare. P resorberas från tunntarmen till blodet, för att därifrån nå ut till olika vävnader, men stora mängder P utsöndras även i saliven. När saliven sedan svalts, passerat våmmen och nått tarmen, kan P från saliven åter resorberas. Denna så kallade recirkulation av P som idisslare har, gör det svårt att bestämma det exakta behovet av P. Dessutom anpassas resorptionen från tarmen efter djurets behov. Mjölkproduktion, fodrets struktur, pH i tunntarmen, djurets egen P-status, mängden utfodrad P i förhållande till behovet, P-källan samt djurets ålder är ytterligare några av de faktorer kan som påverka upptaget av P.

Syftet med den här avhandlingen har varit att öka kunskaperna om P och studera olika faktorer som påverkar kons P-upptag, samt även undersöka hur skelettet utnyttjas såsom P-reserv. För att studera hur mycket P som kon tar upp, beräknades smältbarheten. Den beräknas som skillnaden mellan P i foder och träck, i förhållande till mängden P i fodret.

Resultaten visade att ju mer P korna ätit, desto lägre blev smältbarheten. En låg smältbarhet är oftast ett tecken på att man utfodrat P i överskott. Så gott som all P utsöndrades i träcken, och utsöndringen i urinen var marginell. För att förstå hur mycket P som utsöndrades i träcken kan nämnas att de kor som fick den största mängden, 142 g/dag, utsöndrade mer än 100 g/dag i träcken. Det visar tydligt att kon inte kan hålla kvar mer P i kroppen, än vad som motsvarar behovet. När träcken analyserades för P-innehåll upptäcktes en stor daglig variation i träckutsöndringen, trots att korna fått samma mängd foder varje dag. Förklaringen låg i att korna utsöndrade olika mängder träck, och mängden P verkade vara kopplat till mängden träck. Detta är ett viktigt resultat, eftersom man i många tidigare försök samlat träck under för kort tid, vilket kan ha gett felaktiga resultat.

Smältbarheten av P mellan olika fodermedel; vete, raps, solros/palmkärna och mineralfoder undersöktes också. Resultaten visade att det inte var några skillnader i smältbarheten mellan dessa foder. I Storbritannien och USA har man infört ett system, där man har olika tillgänglighetsfaktorer för olika fodertyper. Men räknar där med att tillgängligheten av P är 64% i grovfoder, 70% i kraftfoder och mellan 30-90% för de olika oorganiska former av P som förekommer i mineralfoder. Det grundar sig på att foderstrukturen har betydelse för upptaget av P. Resultaten från den egna studien tyder på att det i dagsläget inte finns någon grund för att införa olika tillgänglighetsfaktorer för olika kraftfoderkällor.

Skelettet är en i allra högsta grad aktiv vävnad. Uppbyggnad respektive nedbrytning av skelettet pågår genom hela livet, men den varierar bland annat med ålder, eventuell dräktighet och laktation. Det är känt att kalcium frisätts från skelettet i början av en laktation, som en konsekvens av det plötsligt ökande behovet för mjölkproduktion direkt efter kalvning. Senare under laktationen, när mjölkproduktionen avtar, lagras kalcium in i skelettet igen. Om kalcium frisätts från skelettet, så måste även P frisättas och kunna utnyttjas. Det skulle i så fall betyda att vi kunde sänka den rekommenderade mängden P i början av laktationen, och därigenom dra nytta av den naturliga P-frisättningen från skelettet som då sker. Resultaten visar att detta är möjligt. Skelettets nedbrytning och uppbyggnad, studerades genom att mäta speciella markörer som finns i blodet, och som speglar skelettets nedbrytning och uppbyggnad. Det visade sig då att uppbyggnaden av skelettet var som högst under mitten av laktationen, och nedbrytningen var störst direkt efter kalvning. Den största uppbyggnaden av skelettet totalt sett skedde under slutet av laktationen, och det är förmodligen extra viktigt att under den tiden tillgodose kons P-behov. Smältbarheten visade att en lägre P-giva i början av laktationen, gör att P utnyttjas mer effektivt under den perioden.

Det är viktigt att värna om miljön, men likväl måste rekommendationerna av P till våra djur vara fullt tillräckliga för att tillgodose deras behov. De sänkningar av rekommendationerna som nyligen genomförts i till exempel Nederländerna, Danmark och USA, har inte haft som syfte att förbättra djurhälsan, utan att minska negativ inverkan på miljön. Man ändrade rekommendationerna så att de sänktes under hela laktationen. Resultaten i den här avhandlingen visar att vi kan sänka rekommendationerna av P i början av laktationen, genom att dra nytta av den P som frisätts naturligt från skelettet, utan riskera att djurhälsan påverkas negativt.

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