Phosphorous and **Calcium** Utilization and Requirements in **Farm Animals**

Edited by
Dorinha M.S.S. Vitti and Ermias Kebreab

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Contents

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1 General Introduction

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Ever since animal agriculture began there has been a drive to improve the productivity of our livestock for economic and survival reasons. For that reason research into animal production has been concerned largely with this objective. The limits on production in early days of agriculture were determined by the natural regenerative processes of the soil and the cycling of crop and animal wastes in a closed, ecologically sustainable system (Conway and Pretty, 1991). Since the Second World War, a tremendous improvement in agricultural production has been observed. This is mainly due to national and international policies that were favourable to farming such as the European Common Agricultural Policy and major technological advances in inputs and techniques available to farmers.

Intensification of animal production led to a serious imbalance between inputs of nutrients in purchased fertilizers, concentrates and forages, and the respective outputs in milk and meat and nutrient accumulation in the environment. Several surveys (e.g. Sansinena *et al*., 1999; Satter and Wu, 1999; Sink *et al*., 2000; Kebreab *et al*., 2008) have revealed that dairy producers in the United States and Canada routinely include 0.45 to 0.50% dietary P in feed. This is in excess of recommendations by NRC (2001) and in excess of the needs of lactating cows (Wu *et al*., 2001; Valk *et al*., 2002). Castillo *et al*. (2009) reported that 62.5% of California dairies fed P in the amounts 120–200% of NRC requirements to their herd. Similarly, 21 out of 40 commercial dairy farms tested in Merced County, California, had 120–200% of NRC requirements of Ca. None of the farms fed P below requirement and all farms fed iron and manganese over 200% of NRC requirements.

The Canadian Food Inspection Agency (CFIA, 2009) recommends 0.30 and 1.3% P/kg DM as minimum and maximum concentrations in feed for dairy cows, respectively. However, CFIA is proposing to change the recommendations to 0.35 and 0.90%, respectively. For Ca, the corresponding values are 0.40 and 2.5%, with the new proposal ranging between 0.25 and 2% respectively, depending

 Phosphorus and Calcium Utilization and Requirements in Farm Animals (D.M.S.S. Vitti and E. Kebreab)

on the type of cattle. For swine, the CFIA is proposing to cut P recommended maximum values by half to 1% and Ca by 30% to 1.4% for growing animals. Although CFIA is going in the right direction in reducing the maximum macro mineral allowance in diets, the scientific evidence suggests that is should set the limits even lower than proposed. There is a general misconception that diets containing the recommended dietary P for ruminants would have an effect on the reproduction performance of the animals. Lopez *et al*. (2004) compared the reproductive performance of dairy cattle fed the recommended level of dietary P (0.37%) and excess P (0.57%) intake. The authors found that there was no detectable effect of diet on reproductive performance. Most of the P added as a 'safety margin' ends up in the faeces and urine and contributes to nutrient loading. Chapter 8 discusses P requirements of ruminants in detail and Chapter 9 deals with P and Ca requirements of swine and poultry.

Phosphorus is a non-renewable resource and 90% of the demand for P is for food production (Gunther, 2005). Phosphorus is essential for plant growth and it is critical for bone development, growth and productivity of farm animals. Animal diets are routinely supplemented with inorganic P sources such as rock phosphate and agriculture is estimated to use 148 million t of rock phosphate every year (Gunther, 2005). At this rate, Steen (1998) estimates that the global commercial phosphate reserve will be depleted in 50–100 years. Therefore it is essential that P use in agriculture is optimized. To avoid severe repercussions in agricultural productivity, Cordell *et al*. (2009) warned that significant physical and institutional changes need to be implemented.

Availability of P from plants and especially grains is an issue that needs to be understood well in order to calculate requirements and optimize P utilization in farm animals. Dietary P occurs in inorganic and organic compounds with varying degrees of solubilities (Underwood and Suttle, 1999). Cereal-based diets, in contrast to grass, contain a significant proportion of their organic P in the form of phytates (NRC, 2001). Although phytates are extremely insoluble, they are highly available to ruminants due to the presence of phytases (Morse *et al*., 1992). Nevertheless, phytate P can bypass the rumen in undigested grain. Present-day high-producing dairy cows readily consume over 20 kg of DM with a concentrate/roughage ratio of 40:60 (Phipps *et al*., 2000). It is hypothesized that the higher DM intake and correspondingly higher flow rate of digesta may result in increased rumen bypass of phytate P and possibly other phosphates, which increases P output in excreta (Kebreab *et al*., 2005). The authors also showed that diets can influence the nature and solubility of P in the faeces and that it may be possible to improve management of P outputs from dairy farms. Grass silage-based diets, for instance, tend to produce slightly higher overall levels of P output compared with urea-treated whole-crop wheat (WCW) diets (1.71 versus 1.57 t/100 cows/year), although as a proportion of intake relatively higher values were noted on WCW diets. However, WCW diets tend to produce more water-soluble P (0.33 versus 0.68 t/100 cows/year) and only slightly less acid-soluble P than grass silage diets (1.4 versus 1.2 t/100 cows/year). The polluting effect on the environment by the different fractions of P in excreta depends on soil PH, structure, texture and organic matter content (Kebreab *et al*., 2005). Hill *et al*. (2008) simulated P utilization in dairy cows and suggested that it is

critical to achieve a better understanding of phytate digestibility in order to lower the level of P supplementation in dairy diets.

The biggest challenge for utilization of phytates is in non-ruminant animals, due to lack of enzymes that break down phytates. A number of experiments have shown that addition of microbial phytase enzyme enhances the hydrolysis of phytate into inorganic P and other organic P compounds. For example, Yitbarek (2009) investigated the effect of feeding a low P, phytase-supplemented diet on growth and P utilization in growing pigs. A total of 20 weaned piglets were divided into two groups, control (NOPHY) and animals fed diets containing reduced P and supplemented with phytase (PHY). The authors used the Lopez growth function (Lopez *et al*., 2000) to analyse body weight versus age relationships and reported a non-significant difference between the two feeding groups in final weight. The authors expressed body weight as a function of cumulative P intake so that the derivative with respect to cumulative intake represented P efficiency. Through the entire growth period the difference in P efficiency was shown to be significantly different from zero. Pigs on PHY treatment had a better P efficiency and grew faster and to a higher body weight than the control. During peak growth, inorganic P sources make up almost 30% of total P in the diet and, in the last 10 weeks of the grower-finisher pig's life, about 20% of P consumed comes from inorganic P supplementation (Fig. 1.1a) in feed without phytase. After 10 weeks of age, there was no need to supplement the diet with inorganic P in the PHY diet and the savings were enormous. Similarly, Fig. 1.1b shows that an average pig consumes 11 times more inorganic P with the NOPHY diet compared with the PHY diet. On average, 0.13 kg of inorganic P was consumed by a pig on a phytase-supplemented diet during its lifetime. On the contrary, 1.4 kg of inorganic P was consumed by the control group to reach a similar level of growth. The implication for mitigating P pollution, especially in areas where P loading is already a problem, is significant.

Environment

Prior to the 20th century and up until the Second World War, the environment was considered predominantly with regard to its effect on production response (i.e. the animal's environment). For example, how can we minimize the negative effect of heat stress on feed intake? Recently, political and consumer pressures have promoted environmental issues up the research agenda. However, these 'environmental' concerns are in direct contrast to those traditionally concerned with improving animal performance. It is the effect of the production system on the wider environment (local and global) that dominates the concerns of consumers, politicians and, to an increasing extent, the producers themselves. The pressure to deliver sustainable production systems will continue to increase as the demand for animal products continues to grow. The challenge for sustainable agriculture research is to improve productivity in a manner that meets both economic and environmental sustainability criteria.

Extensive animal production regimes are scrutinized for their high greenhouse gas emissions per unit of feed energy input and level of productivity, while

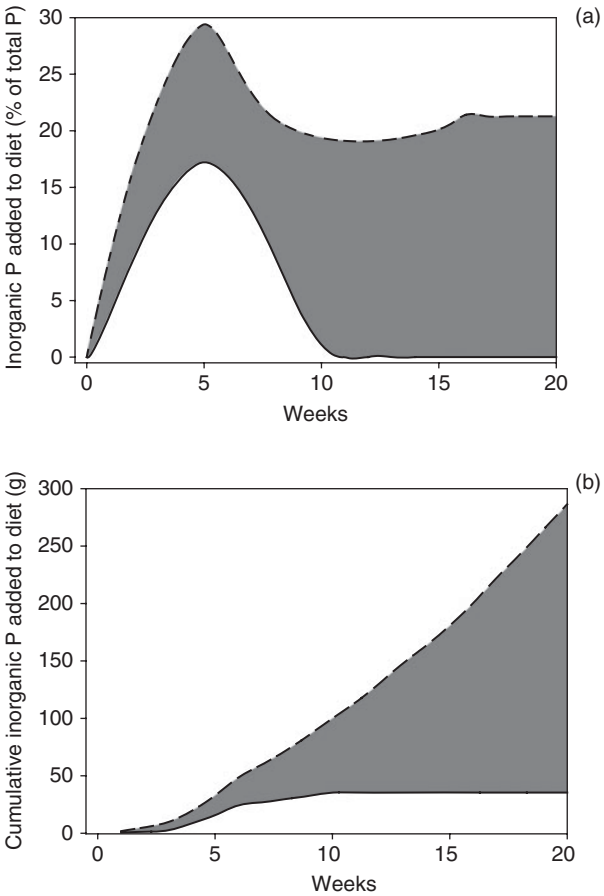

Fig. 1.1. Inorganic P added to grower-finisher diets (a) as a percentage of total P in diet, and (b) accumulated over the pigs' lifespan. Solid and broken lines represent pigs fed diet with or without phytase supplementation, respectively.

intensive systems are criticized for inefficiency in converting nutrient intake into product and the resulting excretion of nutrients to the environment. Those involved in animal production from research to industry often view these concerns with scepticism. However, the objectives of improved production and ameliorating the impact on the environment may not be mutually exclusive. Stated simply, nutrient retention and nutrient pollution (loss) are two sides of the same coin. Therefore, it should be possible to develop a cohesive solution that delivers for producers, consumers and politicians alike. The solution must consider not only methods to maximize nutrient retention but also those that satisfy eating quality, food safety, animal welfare and financial objectives.

Dietary P deficiency is a problem in animal production in many countries. In most tropical countries, dietary P deficiency is one of the predominant problems of mineral imbalance in ruminant production from extensive agricultural systems. Most pastures in Brazil, for example, have low P and as a result productivity from ruminants is low. Therefore, P needs to be supplemented in many forage-based diets but, due to the cost of P and potential environmental pollution, the amount of supplemental P needs to be estimated accurately. The

absence of appropriate P data, especially from kinetic studies of P metabolism, and the complicated nature of P metabolism and its efficiency of utilization in farm animals have led to a variety of estimates of P requirements in ruminants (ARC, 1980; AFRC, 1991; NRC, 2001). In Chapter 7, P nutrition and metabolism are discussed in detail. For example, in ruminants the amount of endogenous P contributing to the total amount of P excreted in faeces can be remarkable. In some instances, the amount of endogenous P excreted in the faeces can exceed that of dietary P. Therefore, accurate determination of the different fraction of P contributing to excreted P is of paramount importance in recommending the amount of P fed to farm animals. The isotopic dilution technique allows accurate measurement of endogenous P and P exchanges between body compartments, and is therefore a suitable technique for studying the fates of P in the animal body. Chapter 2 is devoted to discussions on the application of the dilution isotope technique using radioactive phosphorus (^{32}P) for determining the endogenous P and its true absorption in domestic animals. Chapter 3 takes the study of isotopic dilution technique further and discusses kinetic models for resolving data collected using such a technique by analysing various compartmental models of different complexity.

Policies Affecting Mineral Excretion

Expansion of intensive agriculture, particularly in developed countries, has led to concerns regarding water quality, air quality and odour in the surrounding areas. Therefore, many governments have set up agencies to develop environmental regulations and a means to implement them. For example in Canada, the Canadian Food Inspection Agency is charged with ensuring all ingredients in feed are safe and sets the minimum and maximum levels of mineral inclusion in the diet. However, every province sets its own limits when it comes to reducing P load on farms. For example, the government of Manitoba and Manitoba Conservation regulate the number of animals that can be kept and the amount of manure spread per unit of land in Manitoba, respectively. Currently, the government of Manitoba has implemented a bill that permanently bans building or expanding animal facilities along the Red River Valley, south-eastern Manitoba and the interlake region (between Lake Winnipeg and Lake Manitoba). These regulations were implemented to reduce P loading and contamination of water bodies (MLMMI, 2009). Similarly, Manitoba Conservation implemented a Livestock Manure and Mortalities Management Regulation, which requires producers to test P content of soil and determine the amount of manure that can be applied based on soil test P (Manitoba Conservation, 2009).

In the USA, the Environmental Protection Agency (EPA) is the governmental body that implements environmental regulations such as concentrations of minerals in water bodies. The EPA also implements regulations related to animal feeding operations. Regulations limiting manure application to the P needs of the crop are explicitly allowed in the recently finalized federal Concentrated Animal Feeding Operation regulations to address water pollution in the USA (EPA, 2001). Based on the assessment of risk of nutrient loss, manure is applied

based on N or P requirement of plants to protect water quality. The N:P ratio in manure is significantly lower than the N:P ratios in plants (Heathwaite *et al.*, 2000); therefore, applications based on N pose a risk of P overload on farms. Phosphorus-based limits are now enforced in several states, such as Michigan, Maryland, Virginia and Florida, with several other states considering a switch to P-based limits.

Several countries in the European Union also have regulations to reduce P pollution in the environment. Although details of regulations in various countries in the European Union differ, similar measures have been implemented or at least been considered for implementation. These include: limiting the number of animals kept per unit of available land; limiting the quantity of feed that may be purchased from external sources; and forcing farmers to compare import and export of nutrients into their farm, particularly in the Netherlands (Pfeffer and Hristov, 2005).

The objective of the book is to provide the reader with information regarding P and Ca metabolism, efficiency of utilization, availability and requirement in livestock, and interactions between the animal and the environment.

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2 Isotope Dilution Technique

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Introduction

The utilization of minerals by animals is usually measured through its absorption and retention in the body. Traditionally, mineral absorption has been investigated using the chemical balance technique, which is the difference between intake and faecal content of the element under investigation. However, for phosphorus (P) and calcium (Ca), the balance technique does not provide meaningful data on absorption because of significant intestinal endogenous excretion of the minerals. The mineral present in faeces is made up of endogenous and exogenous sources (Kleiber *et al.*, 1951). The exogenous fraction is the unabsorbed mineral from dietary sources and the endogenous fraction is represented by the mineral which reaches the digestive tract and comes from the body, as part of secretions, digestive juices, cells and saliva. The higher the endogenous excretion as a fraction of intake, the greater is the difference between apparent and true absorption in the conventional balance technique (Van den Berg *et al.*, 1995). Knowledge of the amount of endogenous fraction allows the true absorption to be determined and it is especially important in nutrition studies because these obligatory losses are used in calculation of mineral requirements (Dias *et al.*, 2006a).

The major disadvantage with the conventional balance technique is that the true absorption from a feedstuff or diet cannot be determined, as there is no way to distinguish in the faecal samples between non-absorbed elements of dietary origin and the same elements excreted from endogenous sources (Sandström *et al.*, 1993). Furthermore, exogenous and endogenous components need to be distinguished because their separation is essential to the quantification of true availability of the mineral (Kirchgessner *et al.*, 1994). The use of isotopes to label dietary minerals and trace elements allows studies of absorption from both individual and total mixed ration diets and is also an appropriate means to study mineral metabolism and factors that influence it (Sandström *et al.*, 1993; Windisch

et al., 1999). In the last few decades, several techniques employing radioactive or stable isotopes have been developed, e.g. the 'comparative balance method', the 'dual tracer' or 'double isotope technique', methods based on computerized compartmental analysis and the 'isotope dilution technique' (e.g. Aubert *et al.*, 1963; Thompson, 1965; Belshaw *et al.*, 1974; Gibson *et al.*, 1988). Among these methods, the isotope dilution technique, in particular, comprises direct and quantitative measurements of mineral fluxes and provides robust estimates of endogenous faecal excretions, as has been shown for a series of macro-minerals and trace elements (e.g. Weigand and Kirchgessner, 1976; Kreuzer and Kirchgessner, 1991; Gabler *et al.*, 1997; Windisch *et al.*, 1999).

This chapter focuses on the application of the radioisotope dilution technique using radioactive phosphorus (^{32}P) for determining the endogenous P and its true absorption in domestic animals.

Basic Principles and Use of the Radioisotope Dilution Technique

The isotope dilution technique involves incorporating an accurately known amount of stable or radioactive isotope (tracer) of a substance to be studied in a sample or a system, isolating part of the substance and determining the isotopic content. An ideal tracer has the same physical or chemical or biological properties of interest as the element of study (trace), but presents some peculiar characteristic that enables its detection in the system where the trace is also present (Cantone and Giussani, 2001). The dilution that occurs is a function of the substance present in the original material (Comar, 1955). Mass differences of isotopes are due to different numbers of nuclear neutrons, so that the chemical properties are not affected. The basic assumption of the technique is that the added isotope of the element or the tracer will not be discriminated from the unlabelled or trace and will trace the position or movement of the unlabelled molecules. In other words, the tracer and sample are in full equilibrium in the system of interest. Either stable (naturally occurring) or radioactive isotopes can be tracers.

Stable isotopes

Stable isotopes were the first tracers to be used in research (Schoenheimer and Rittenberg, 1935) and are valuable tools in mineral availability and metabolism studies. However, their use as tracers has some limitations. In order to be accurately measured, stable isotopes often have to be added at levels that substantially increase the total element intake (Sandström *et al.*, 1993). Some minerals have just one stable isotope and others have abundant isotopes. For example, P has only one naturally occurring isotope and, because stable isotopes cannot be enriched, stable isotope tracer studies cannot be conducted. Other minerals, such as copper and magnesium, have abundant isotopes; therefore, as the abundances of isotopes increase, the amount that must be administered increases and quantities greater than true tracer doses are often required. This introduces a risk of perturbing metabolism with the doses required for adequate enrichment. In

addition, another limitation of the use of stable isotopes as tracers could be the cost of analysis. Sample preparation can be laborious and high-precision analytical methods are required, which can be very expensive (Turnlund, 2006). Calcium has more than five naturally occurring isotopes, providing a wide variety of opportunities for metabolic studies. However, the half-life of ^{45}Ca may be too long in relation to nutritional studies. The biggest advantage of using stable isotopes is the safety factor. Therefore, they are often used in studies of mineral metabolism in human subjects (e.g. Turnlund *et al*., 2003), particularly pregnant women and newborns. The isotopes can be used as tracers with no exposure to radioactivity and they do not decay, so long-term studies can be conducted and sample analysis can be delayed. Moreover, some minerals have no radioisotope that can be used satisfactorily as a tracer.

Radioisotopes

Isotope dilution technique with the use of radioisotopes was first introduced by Hevesy and Hobbie (1932) in chemical studies. Many variations of the basic technique have since been developed. Radioactive isotopes have played and still continue to play a key role in the understanding of metabolic aspects in cells or bacteria, yeasts, plants and animals, including humans, and in the elucidation of the fundamental properties of genetic material (Cantone and Giussani, 2001). When radioisotopes are used, one of the primary limitations is exposure to radiation. In addition, some minerals have no suitable radioisotope for tracer studies. The radioisotope half-life must be taken into account, and it needs to be long enough to run the experiment, and not too long, causing problems of disposal and storage. Other limitations to be considered are type of radiation emitted by the radioisotope and its energy, ease of measurements and radioactive exposure of the researchers working in laboratories (IAEA, 1979).

The advantages of the use of radioisotopes as tracers are that radio nuclides can be measured accurately with high sensitivity even in small amounts and there are a variety of labelled compounds commercially available. Radioisotopes can be measured by rapid and simple methods of analysis and little sample preparation is usually necessary. Instrumentation used to detect the radioisotopes is considerably less expensive than that used for stable tracers, and the isotopes themselves are usually less expensive. As a result, studies with radioisotopes are less costly and can be completed more quickly. In addition, radioisotopes can be localized *in vivo*.

The use of radioisotopes to determine the endogenous loss of an element and its absorption was emphasized by Hevesy (1948). Kleiber *et al.* (1951), who worked with ^{32}P, proposed a methodology to determine the endogenous faecal P and the true digestibility in studies with ruminants, and later Visek *et al.* (1953) applied a similar method to study calcium (Ca) absorption, using radioactive calcium (^{45}Ca). Improved methods for determination of endogenous P were developed by Lofgreen and Kleiber (1954; Fig. 2.1) and Luick and Lofgreen (1957). The researchers injected the isotope subcutaneously to avoid the rapid disappearance of intravenously injected ^{32}P and used the radioisotope in insoluble

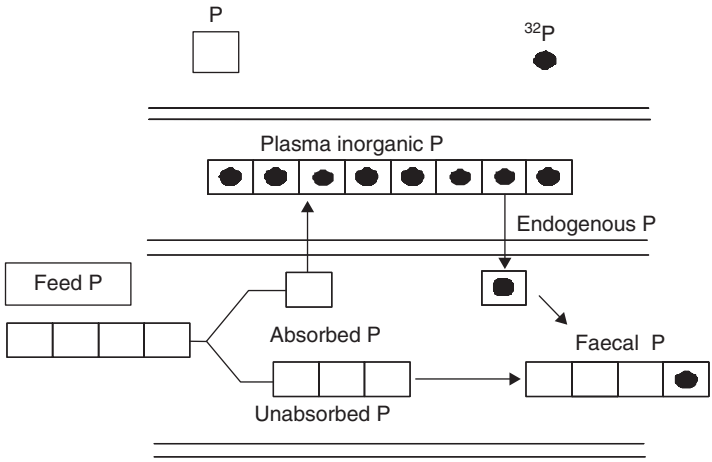

Fig. 2.1. A scheme to illustrate the isotope dilution technique for determination of endogenous P using a parenteral injection of ^{32}P (adapted from Lofgreen and Kleiber, 1953).

salts, such as tricalcium-phosphate. Subsequently, other experiments were carried out using ^{32}P and ^{45}Ca with sheep and cattle for determination of endogenous P and the factors affecting it. Studies with rats (Whittemore *et al*., 1973) using ^{32}P showed that the level of P in the diet could affect the endogenous loss. An increase in the rate of excretion of endogenous P was observed when adequate P was given to sheep which had been previously depleted, and animals fed a diet with a low P level showed a decrease in endogenous P (Young *et al.*, 1966). Other studies with lambs fed different P levels showed that there was a linear relationship between P intake and endogenous P loss (Braithwaite, 1985; Ternouth, 1989). More recent studies have been done with ruminants and non-ruminants (Vitti *et al.*, 2000; Dias *et al.*, 2006a, 2007; Louvandini and Vitti, 2007), using ^{32}P and ^{45}Ca to study the kinetics of these minerals in the animal body.

The underlying principle behind the isotope dilution technique is the conservation of mass upon dilution. In the radioisotope dilution method the conservation of mass is manifested by conservation of activity. The dilution of the radioactive isotope by its non-radioactive counterpart results in the reduction of specific activity, defined as the activity per unit volume or mass, in a conserved manner proportional to the original specific activity and the amount of analyte (Fassett, 1995). Labelled P in faeces shows up in a very short time after its introduction through injection of radioactive P (Annenkov, 1982). Stable and radioactive isotopes behave in an identical manner and enter the digestive tract in the same proportion. However, when the labelled element is secreted into the digestive tract, it is diluted with the unabsorbed mineral from the diet (exogenous). The extent of dilution is calculated by the ratio of faecal and plasma specific activities and the endogenous fraction of mineral is determined according to eqns 2.1–2.7

(Comar *et al*., 1953). Higher specific activity indicates a higher endogenous fraction. If the mineral ingestion and the total amount of the mineral in faeces are determined, the absolute amount of endogenous element in faeces and the true absorption of the mineral can be calculated.

^{32}P is very convenient because its half-life is 14.3 days, which is not too short but is long enough to run an experiment. Another advantage is that ^{32}P is a β emitter, with maximum energy of 1.71 MeV, which allows the use of the Cerenkov effect for detection of radiation. This permits the use of water as a medium for diluting the samples and there is no need for scintillation solutions, making the analysis much simpler and avoiding disposal problems and chemical pollution. The procedures for the isotope dilution technique using ^{32}P to determine endogenous P and true absorption (Kleiber *et al.*, 1951; Lofgreen and Kleiber, 1953; IAEA, 1979; Vitti, 1989) are summarized as follows:

1. The animal is kept in metabolic cages to allow quantitative collection of faeces and urine and to control feed intake. During an adaptation period, the animal is conditioned to consume the experimental diet normally.
2. When the intake and excretion are stabilized, showing that the animal is reacting normally, the animal is injected (intravenously, subcutaneously or intramuscularly) with a carrier-free solution of disodium hydrogen (^{32}P) orthophosphate diluted in saline (0.85%) solution. The dose applied depends on the animal species of interest. For example, in sheep and pigs of about 40 kg live weight or less, 7.4 MBq of ^{32}P is enough. For cattle and horses the adequate dose is about 30 MBq ^{32}P.
3. During 7–8 days, feed intake and excretion are recorded daily and for each day a representative faecal (10%) sample is taken for analysis. Blood samples are collected during the period.
4. The mineral content in the food is analysed (by colorimetric methods) to calculate the total mineral consumption. Blood and faeces samples are analysed for total and radioactive P. Radioactivity is measured in a liquid scintillation counter and corrections for quenching and decay are made by the external standard technique (external source channel ratio method) (Nascimento Filho and Lobão, 1977). A schematic illustration of the method is given in Fig. 2.2.

Endogenous P and true absorption are calculated from specific activities of plasma and faeces. Counting of samples (corrected for background and radioisotopes decay) is expressed as a percentage of administered dose. For the calculations, the data of specific activities versus time are fitted to an exponential curve. At the third or fourth day from injection, it is expected that the slope of the plasma curve will be parallel to the slope of the faecal curve, indicating that there is an isotopic equilibrium. Counting values when plasma and faecal counting is at equilibrium are considered for the calculations. It is also important to know the time interval between the injection and the reappearance of measurable quantities of radioactive P in faeces (Lofgreen *et al.*, 1952). This depends on the animal species and is normally between 24 and 48 h. The specific activities of the faecal and plasma samples at various intervals are measured and then the time lag from the initial injection of ^{32}P in plasma to the excretion in faeces is estimated. For

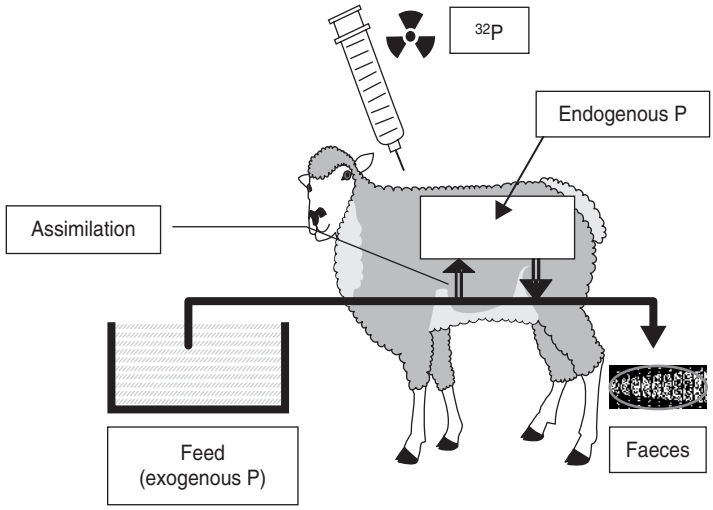

Fig. 2.2. A schematic representation of the radioisotope technique in sheep.

sheep, it is assumed to be a period of 24 h. The calculation of the endogenous P and true absorption is as follows:

Plasma:

$$\text{Injected activity (\%)} = \frac{\text{counting per minute of sample}}{\text{counting per minute of standard}} \times 100 \quad (2.1)$$

$$\text{Specific activity in plasma} = \frac{\text{injected activity (\%)}}{\text{plasma (mg P/ml)}} \quad (2.2)$$

Faeces:

$$\text{Specific activity in faeces} = \frac{\text{injected activity (\%)}}{\text{faeces (mg P/g)}} \quad (2.3)$$

Endogenous P loss (%) is calculated from the average of the specific activity values for faeces and plasma as follows:

$$\text{Endogenous P (\%)} = \frac{\text{specific activity in faeces}}{\text{specific activity in plasma}} \times 100 \quad (2.4)$$

Total faecal endogenous P is calculated based on the daily faecal P excretion as follows:

$$\text{Total faecal endogenous P (g/day)} = \text{endogenous faecal P (\%)} \times \text{faecal P output (g/day)} \quad (2.5)$$

The true absorption of dietary P is:

$$\text{True P absorption (g/day)} = \text{P intake (g/day)} - [(\text{exogenous P (g/day)} - \text{endogenous P (g/day)}] \quad (2.6)$$

The efficiency of P absorption, which indicates the absorption as a fraction of the dietary P intake, is:

$$\text{Efficiency of P absorption} = \frac{\text{absorbed dietary P (g/day)}}{\text{P intake (g/day)}} \quad (2.7)$$

Administration of Radioactive Solution and Handling of Radioactive Material Injected Animals

Preparation and administration of radioactive solution

Proper use of equipment, techniques and procedures allows the use of radioisotope with minimal exposure for the workers and avoids extensive contamination of the facilities and equipments. Control of radioactive exposure is based on the assumption that any exposure involves some risk. The workers must keep it in mind to maintain exposure as low as can reasonably be achieved. Laboratory personnel involved in experiments using live animals and radionuclides must have specific training in safe use of radioactive materials and animal care.

In designing and planning mineral nutrition experiments with radioisotopes using animals, it is essential to understand the metabolism of the element in the study. The investigators need to have information about the characteristics of the radionuclide preparation, with regard to chemical or radiochemical impurities that may be present. It is necessary that the solution be suitable for administration to the animals regarding pH level and freedom from particulate material (IAEA, 1979). The application of the radionuclide may not cause problems in the normal physiology of the animals. The researchers have to consider the amount of the radioisotope to be administrated based on the half-life, energy of the emitted radiation, taking the animal weight into account, the duration of the experiment and the time required for analysis. These are important factors to assure the sensitivity, precision and accuracy of the method.

In most studies it is necessary to administer the radionuclide to the animal quantitatively. Methods for the radioisotope introduction into the animal body can be via oral, intravenous, subcutaneous, intramuscular and intraperitoneal injection. It is usually necessary to prepare a standard solution. This should be done from the original preparation in such a way that the amount administered to the animal can be exactly correlated with the amount used to make up the standard solution (IAEA, 1979). It is important that healthy animals are used and maintained during the study. The animal should be preconditioned to the type of handling and management during the experimental period. Animals to be used in *in vivo* studies with radioisotopes must be housed in a separate room specifically designated for that purpose by the radiation safety officer. After the administration of radionuclides, the animal must be considered as a source of external radiation and of radioactive material excreted and expired, which can contaminate surroundings. Provision should be made for appropriate collection and disposition of radioactive excretion.

Procedures for handling animals injected with radioactive materials

The proper collection of samples is extremely important and it is essential that collection is complete or representative of the material under study. In studies where the entire sample is not used, appropriate aliquots must be taken and thoroughly mixed. For example, care must be taken in mixing faeces well before sampling because successive increments may differ widely in activity during the experimental period. Special care has to be taken if grinding dry samples is necessary.

The form of the samples required for counting will be determined primarily by the type and energy of radiation emitted. With gamma-ray emitters it is possible to count solid samples directly. β emitters, such as ^{32}P and ^{45}Ca, frequently need sample preparation, such as oxidation and ashing. Liquid samples, such as blood, plasma and urine, may be assayed directly by liquid counting. Liquid scintillation detectors are often used because they are cheap, efficient and robust (IAEA, 1979).

Although new techniques and methodologies are available at present to study mineral metabolism, the isotope dilution technique based on the work of Lofgreen and Kleiber (1953, 1954) is still used in animal science research and continues to provide essential data. Together with tracer kinetic modelling (discussed in Chapter 3), the radioisotope dilution technique has been used to develop more complex mathematical models to study mineral metabolism (e.g. Fernandez, 1995; Vitti *et al.*, 2000; Kebreab *et al.*, 2004; Dias *et al.*, 2006b). Estimates of endogenous P and Ca requirements for various species have been calculated using the technique (e.g. Vitti *et al.*, 2000, for goats; Dias *et al.*, 2006a, for sheep; Dias *et al.*, 2008, for swine). Chapter 4 discusses the use of the isotope technique in detail with specific minerals and species.

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3 Kinetic Models for the Study of Phosphorus Metabolism in Ruminants and Monogastrics

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Introduction

Phosphorus (P) is an essential macro-mineral for all animals. This nutrient is necessary for many body functions, fundamental to maintenance and repair of all body tissues and indispensable, along with calcium and other minerals, for proper growth and mineralization of osteoid tissue and muscle development. Sufficient P intake is important throughout life to ensure the proper balance of essential minerals and allow for adequate feed and energy utilization. Phosphorus enhances reproductive performance at several stages in the reproductive cycle, and adequate P is important to achieve high rates of milk and egg production.

Deficiency of P is the most widespread and economically important of all the mineral deficiencies in farm animals, affecting feed consumption and animal performance, so that supplemental dietary P is needed under most practical feeding situations. Thus, it is important to determine phosphorus requirements that ensure animal health and productivity accurately, while minimizing nutrient excretion in manure.

However, the study of P metabolism and of the efficiency of utilization of this mineral in farm animals is not so straightforward. For instance, in ruminants the amount of endogenous P contributing to the total amount of P excreted in faeces can be remarkable. In some instances, the amount of endogenous P excreted in the faeces exceeds that of dietary P (Kleiber *et al.*, 1951). This occurs because ruminants produce a large volume of saliva, which has an important role as a buffer against volatile fatty acids produced in the rumen. In addition, it has been shown that the salivary glands play a major role in P homeostasis. Due to the considerable contribution of endogenous P to total faecal P output, it is essential to measure this P fraction when determining P digestibility and studying P

metabolism in ruminants. Although in monogastrics endogenous P in faeces is proportionally less than in ruminants, it still affects the results from P digestibility and metabolism studies. Measurement of endogenous P cannot be accomplished straightforwardly since both P fractions, endogenous and dietary, are mixed in faeces. Therefore, a technique capable of separating out these two fractions is required.

The isotopic dilution technique allows accurate measurement of endogenous P and P exchanges between body compartments, and is therefore a suitable technique for studying the fates of P in the animal body. The main isotope used with this technique is ^{32}P, which is a heavy radioactive isotope with a mass number of 32 and a half-life of 14.3 days. This radioisotope is produced in nuclear reactors and emits a β particle, which is detectable by scintillation counters. Radioactivity may be expressed as decay or disintegrations per minute (dpm), i.e. the number of atoms in a given quantity of radioactive material that have decayed in 1 minute. The usual way to detect radioactivity from animal samples is through a liquid scintillation counter, which gives values in counts per minute (cpm), i.e. the number of atoms in a given quantity of radioactive material that are detected to have decayed in 1 minute. The unit dpm is calculated from the measured cpm, corrected for inefficiency of counting (Wolfe and Chinkes, 2005). A standard solution containing a known number of dpm is used to calibrate the counter for determining its efficiency. Usually the standard solution is a sample of the solution injected into the animal. Currently, the becquerel (Bq) and the curie (Ci) are the recommended official units of radioactivity. One Bq is equal to one disintegration per second, or 60 dpm. One Ci is equal to 3.7×10^{10} Bq, equivalent to 2.22×10^{12} dpm. For isotopic dilution studies it is important to determine the specific activity (*s*) or specific radioactivity (SRA) as the way for expressing the abundance of a radioactive tracer. Specific activity refers to the radioactivity or disintegration rate per unit mass of P, which is equivalent to the tracer to tracee ratio and reflects the mixture of non-radioactive and radioactive isotopes. This is because there is radioactivity only in the tracer, and the amount of unlabelled substrate given with the tracer is insignificant.

One of the first studies to employ the isotopic dilution technique to determine P digestibility in ruminants was by Kleiber *et al.* (1951), who estimated the amount of endogenous P excreted in the faeces of dairy cows and calculated the true digestibility of P in the diet. Subsequently, researchers used ^{32}P to trace the mineral in the body of the animal to study its metabolism through exchanges between compartments such as bone and soft tissues. In these studies, it is assumed that radioactive tracer (^{32}P) mixes completely with unlabelled P as represented by dietary (exogenous) P. Usually samples of blood and faeces are collected over 7 days, commencing from the day when tracer is injected, and it is assumed that the last 2–3 days represent a period of non-isotopic steady state. Other samples, such as saliva and rumen fluid, may also be collected when the animals are in this steady-state period, and tissue samples may likewise be used for P measurements after the animals have been sacrificed. This valuable technique is necessary for the development of compartmental models used to understand P metabolism in ruminants and monogastric animals.

Phosphorus Metabolism

Most of the P in animals (approximately 80%) occurs as a constituent of bone. The other 20% occurs in various organic compounds that play key roles in metabolism (e.g. ATP, creatinine, enzymes), in nucleic acids (e.g. DNA, RNA) and in membrane phospholipids. Inorganic phosphate participates in buffering the pH of biological fluids.

Dietary P is absorbed in the phosphate form from the small intestine. It is necessary that the phosphate be in solution at the point of contact with the intestinal mucosa, as any compound forming an insoluble complex with the phosphate ion will decrease its absorbability. Large amounts of calcium (Ca) can cause the formation of such insoluble salts, reducing P absorption; therefore the ratio Ca:P of a feed is an important factor affecting P absorption, at least in non-ruminant species. Phosphorus contained in plant phytates cannot be completely extracted, and only a variable fraction of the mineral can be absorbed. Absorbed P is rapidly transferred into skeletal and soft tissues of the body. There is continuous turnover of P in the body, so resorption from bone and return from soft tissues take place concurrently with accretion (i.e. synthesis). Bones serve as storehouses of P that can be mobilized at times when P absorption is inadequate to meet body needs. Therefore, P metabolism involves not only deposition of the mineral, but also processes of storage and mobilization. Depletion of reserves involves no physiological harm, as these can be readily restored with an adequate diet during periods when requirements are lower. Finally, P is lost from the body by way of faeces and urine in all species, and also by perspiration in those species exhibiting profuse sweating. Faeces are the principal path of P excretion in herbivores, whereas urine is the principal path in carnivores, and both paths have about the same importance in other species (e.g. humans). In ruminants, urinary P output can be considered almost negligible.

Phosphorus accretion by the skeleton increases over the range of dietary P supply from deficient to adequate and shows only a very small response to further increase in P intake (excessive), whereas P resorption from the skeleton remains practically unaffected by P intake. The kinetic changes due to increasing provision of dietary P are increases in faecal and urinary P excretion and, to a lesser extent, in P retention. Hence P concentration in body weight gain can vary depending on P intake. Other outputs of P are in products such as milk or eggs. In lactating animals and laying hens, P utilization for milk or egg production has a priority over other functions, so it is mobilized from reserves to meet these demands when dietary provision is insufficient. In the gut, there are important endogenous secretions of P that reach the intestinal contents by diffusion from plasma or originate from digestive secretions such as saliva and intestinal tissue (cells sloughed off from mucosa) (Pfeffer *et al.*, 2005). As a consequence, the amount of P entering the intestine greatly exceeds P intake. For example, in ruminants the quantity of P secreted into the gut via saliva is generally greater than the quantity present in the feed. Some of this P of endogenous origin can be reabsorbed, but the remainder appears in faeces with the undigested dietary P. Dietary and endogenous P (from saliva or other sources) is mixed completely in the gut, and P absorption occurs mostly from the small intestine without differentiation between endogenous and dietary origin.

Faecal P contains three fractions: undigested dietary P, inevitable faecal P losses and surplus P excreted to maintain homeostasis (Pfeffer *et al.*, 2005). The undigested fraction is from P bound in feed compounds, such as phytates, so that it cannot be absorbed. This fraction can be considered insignificant in ruminants owing to the ruminal breakdown of such compounds by microbial phytases, but in non-ruminant species differences in availability of dietary P among different P sources are of great relevance. The inevitable losses derive from animal metabolism and are obligatory to normal basal functions, thus representing the predominant components of maintenance of the animal. Finally, further P is excreted when the amount of P absorbed exceeds that needed for inevitable losses and for growth, reproduction or lactation in order to maintain homeostasis. In ruminants this surplus is excreted in faeces, whereas in non-ruminants urinary excretion of this fraction becomes more relevant as P intake increases.

In quantitative nutrition, it is of special interest to define and determine measures of the efficiency of utilization of nutrients, in this case of dietary P. Given the considerable contribution of endogenous P to total faecal P output, there is consensus that the apparent digestibility coefficient of P (and of minerals generally) has little significance. However, there has been less agreement as to what constitutes 'utilization' and how it should be expressed. Therefore, a number of terms have been used (availability (true and apparent), utilization, digestibility (true and apparent), absorption, net absorption, retention, etc.), leading to some confusion. As an example, the term 'availability' has been commonly used as synonymous with true digestibility, but also to indicate the joint efficiency of digestive and metabolic utilization of the mineral (mainly because urinary losses in some species, such as ruminants, can be considered insignificant). Similarly, the term 'net absorption' has been used to denote the amount of P which remains in the body ('apparent retention'), as determined in balance trials. We advocate the terminology proposed by Thompson (1965). According to this author, if I is P intake, F total faecal P excretion, $F^{(e)}$ endogenous faecal P excretion, U total urinary P output and $U^{(e)}$ endogenous urinary P output (all in units of g/day), then the following measures of efficiency of P utilization can be defined:

Apparently absorbed P (g/day) $= I - F$
Apparent digestibility (g absorbed per g ingested) $= (I - F)/I$

Truly absorbed P (g/day) $= I - (F - F^{(e)})$
True digestibility (g absorbed per g ingested) $= [I - (F - F^{(e)})]/I$

Apparently available P (g/day) $= I - (F + U)$

Truly available P (g/day) $= I - (F - F^{(e)}) - (U - U^{(e)})$
True availability (g retained per g ingested) $= [I - (F - F^{(e)}) - (U - U^{(e)})]/I$

Availability can be defined as that proportion of a nutrient source provided in the feed which, at a stated concentration and level of feeding, can be extracted, absorbed and used by the animal to meet its requirements. In principle, it accounts for efficiency of digestive utilization of ingested P and also of metabolic utilization of absorbed P in the tissues. It is synonymous with true digestibility when the amount of the element excreted in urine is only of endogenous origin.

It is reasonable to assume that, in the region of negative to zero P retention, U will approximate to $U^{(e)}$, and therefore only when high dietary intakes result in some significant urinary excretion will the two terms materially differ. The magnitude of such differences will depend upon the partition of the excreted P between urine and faeces in the particular animal species under study. In the ruminant, excretion in urine is virtually negligible compared with that in the faeces. As apparent digestibility and retention take no account of endogenous losses, dietary P intakes below maintenance commonly result in negative values, which are clearly unacceptable as expressions of dietary utilization. True digestibility and availability clearly have limiting values of zero.

Regression of faecal output on intake allows calculation of endogenous faecal P (extrapolated intercept for intake = 0) and true digestibility (from the estimate of slope). Endogenous faecal P ($F^{(e)}$, g/day) can be calculated from isotopic data (see next section), using:

$$\frac{\text{specific activity in faeces}}{\text{specific activity in plasma}} = \frac{F^{(e)}\,(\text{i.e. endogenous faecal P})}{F\,(\text{i.e. total faecal P})}$$

In ruminants, it can be assumed generally that 0.65–0.75 of faecal P is of endogenous origin. In a regression of P retention against P intake, the intercept represents total endogenous output, whereas the slope represents true availability. However, this relationship is actually curvilinear, as the efficiency of utilization of dietary P decreases as P intake increases, according to a diminishing returns pattern.

Modelling Phosphorus Metabolism in Farm Animals

Experimental data obtained in tracer studies need to be resolved using compartmental modelling in order to quantify the amount of P present in different parts of the animal (pool sizes), the rates of exchange of P between pools (flow rates) and the loss and net utilization of dietary P (efficiency of utilization). A number of models have been proposed to represent P metabolism in animals, and those that have received most attention will be reviewed in the following sections.

Two-pool Models

Lofgreen and Kleiber (1953)

The scheme assumed by Lofgreen and Kleiber (1953) is depicted in Fig. 3.1. It consists of two pools, gut lumen P and plasma P (both in g of P). There are three flows of primary interest, namely dietary intake of P (F_{10}), flow of P from plasma to the gut (F_{12}) and excretion of P in the faeces (F_{01}). All flows are in units of g/day. Radioactive phosphorus, ^{32}P, is administered by serial injection into systemic blood (four times daily) and the specific activities of pools 1 and 2 monitored. Let these specific activities be denoted by s_1 and s_2 (both μCi/g),

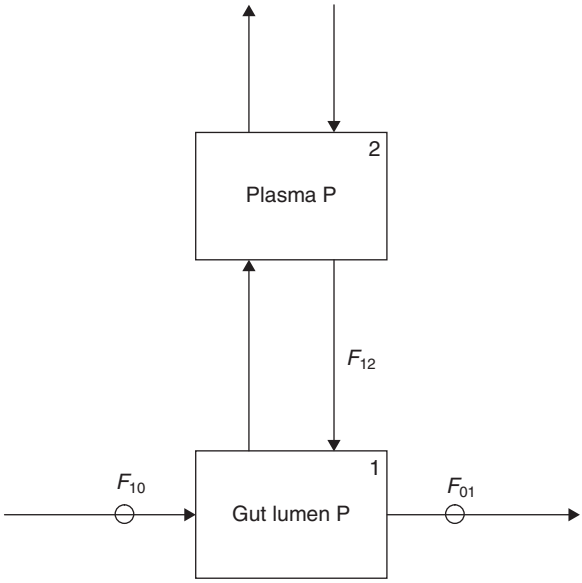

Fig. 3.1. The two-pool model of Lofgreen and Kleiber. Flows between pools and into and out of the system are represented by arrows, with a small circle indicating a measured flow. Adapted from Lofgreen and Kleiber (1953).

respectively (mathematical notation is defined in Table 3.1). Conservation of mass principles applied to the rumen ^{32}P pool give the following differential equation:

$$\frac{dq_1}{dt} = s_2F_{12} - s_1\left(F_{01} + F_{21}\right)$$

where q_1 is the size (μCi) of the pool and t is time post first injection (days). If the gut lumen ^{32}P pool is in a steady state, then dq_1/dt is zero and:

$$s_2F_{12} = s_1F_{01} + s_1F_{21}$$

Assume further that no label entering the gut lumen is recycled to the plasma pool, only voided in the faeces. The second term on the right-hand side is then zero, giving:

$$s_2F_{12} = s_1F_{01}$$

Therefore:

$$F_{12} = \frac{s_1}{s_2}F_{01}$$

i.e. the ratio s_1/s_2 gives the proportion of the faecal P which is of metabolic origin. Some of the values for this ratio reported in the literature are shown in Table 3.2.

Smith *et al.* (1955)

The scheme of Smith *et al.* (1955) for ruminants, shown in Fig. 3.2, is essentially a renomination of that proposed by Lofgreen and Kleiber (1953). The flows of

Table 3.1. Principal symbols used for the kinetic models.

D	Dose of ^{32}P administered to plasma at time zero	μCi
F_{ij}	Flow of P to pool i from j; F_{i0} denotes an external flow into pool i and F_{0j} denotes a flow from pool j out of the system	g/day
f_{ij}	Flow of ^{32}P to pool i from j; f_{i0} denotes an external flow into pool i and f_{0j} denotes a flow from pool j out of the system	μCi/day
Q_i	Quantity of P in pool i	g
q_i	Quantity of ^{32}P in pool i	μCi
s_i	Specific radioactivity of ^{32}P in pool i (= q_i/Q_i)	μCi/g
t	Time since (first) injection	days

primary interest are dietary intake of P (F_{10}), secretion of P into the rumen (F_{12}), its absorption across the rumen wall (F_{21}) and outflow from the rumen to the lower gastrointestinal tract (F_{01}). Conservation of mass principles applied to the rumen P pool give the following differential equation:

$$\frac{dQ_1}{dt} = F_{10} + F_{12} - F_{01} - F_{21}$$

where Q_1 is the size (g) of the pool and t is time (days). If the rumen P pool is in a non-isotopic steady state, then Q_1 is a constant, dQ_1/dt is zero and:

$$F_{01} + F_{21} = F_{10} + F_{12}$$

A single dose of ^{32}P is administered at time $t = 0$ by injection into the jugular vein and the specific activities of the rumen P and plasma P pools (s_1 and s_2, respectively) monitored. Instantaneous ^{32}P secretion into the rumen is given by the product s_2F_{12} and:

$$^{32}\text{P secreted in the time interval } (0,t) = \int_0^t s_2F_{12}dt \tag{3.1}$$

Instantaneous outflow of ^{32}P from the rumen is $s_1(F_{01} + F_{21})$ and:

$$^{32}\text{P outflow over the time interval } (0,t) = \int_0^t s_1\left(F_{01} + F_{21}\right)dt \tag{3.2a}$$

$$= \int_0^t s_1\left(F_{10} + F_{12}\right)dt \tag{3.2b}$$

The amount of ^{32}P in the rumen at time t (i.e. s_1Q_1) is given by subtracting Eqn 3.2b from 3.1:

$$s_1Q_1 = {}^{32}\text{P secreted in interval } (0,t) \tag{3.3a}$$

$$- {}^{32}\text{P outflow over interval } (0,t)$$

$$= \int_0^t s_2F_{12}dt - \int_0^t s_1\left(F_{10} + F_{12}\right)dt \tag{3.3b}$$

Table 3.2. Intake and excretion of P and the ratio of specific activities in faeces (s_1) to plasma (s_2) in different animals.

Animals	Description	Live-weight (kg)	Dietary P (g/kg DM)	Ingested P (g/day)	Excreted P (g/day)	Ratio (s_1/s_2)	Reference
Cows	Milk yield 9.8 kg/day	427	3.5	37.1	32.5	0.43	Kleiber *et al.* (1951)
Cows	Milk yield 9.8 kg/day	430	3.5	12.5	14.5	0.70	Kleiber *et al.* (1951)
Lambs	Wethers	31–36	2.5	2.31–2.49	1.69–1.98	0.75–0.95	Lofgreen and Kleiber (1953)
Lambs	8 months old	31.6	4.8	3.19	4.47	0.53	Dias *et al.* (2006)
Lambs	5 months old	23	1.1–5.6	0.91–6.59	1.4–5.5	0.48–0.73	Dias *et al.* (2007)
Goats	Castrates 4–5 months old	26–29	0.75–3.8	0.42–3.63	0.53–2.03	0.28–0.44	Vitti *et al.* (2000)
Horses	10 months old	257	9.0–10.3	23.2–26.3	18.5–20.7	0.11–0.14	Lopes *et al.* (2003a)
Horses	19 months old	323	2.2–5.0	10.6–25.3	5.4–18.4	0.17–0.37	Lopes *et al.* (2003b)
Pigs	Castrates	28.5	3.2	3.60	2.12	0.11	Teixeira *et al.* (2004)
Pigs	Castrates	28–30	5.6	6.94–7.34	2.41–3.52	0.07–0.10	Teixeira *et al.* (2004)
Pigs	Castrates	32	7.4	9.6–9.8	5.8–7.30	0.04–0.06	Moreira *et al.* (2004)
Pigs	Castrates	19.5	2.7–6.5	1.88–5.63	1.25–2.35	0.10–0.18	Schulin-Zeuthen *et al.* (2005)

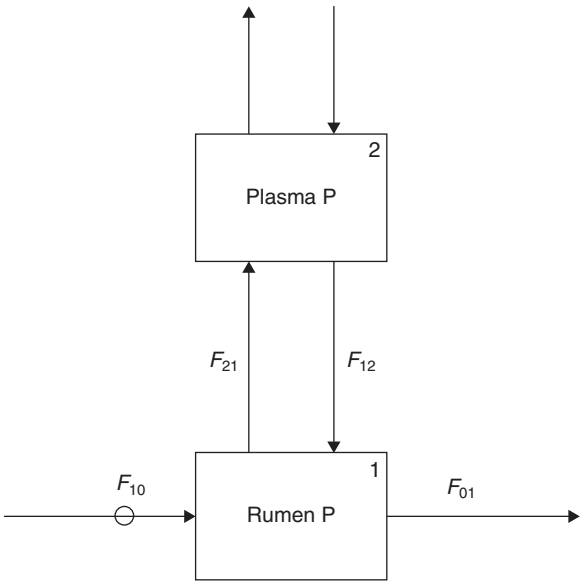

Fig. 3.2. The two-pool scheme of Smith *et al*. Arrows represent flows between pools and into and out of the system, with a small circle indicating a measured flow. Adapted from Smith *et al*. (1955).

Algebraic manipulation of Eqn 3.3b gives F_{12}, the rate of secretion of P into the rumen:

$$F_{12} = \frac{s_1\tilde{Q}_1 + F_{10}\int_0^t s_1 \mathrm{d}t}{\int_0^t s_2 \mathrm{d}t - \int_0^t s_1 \mathrm{d}t}$$

The tilde above Q_1 indicates that the size of the rumen P pool has to be measured experimentally or estimated. Application of the model is illustrated in Table 3.3.

A Three-compartment Model

Fernández (1995)

The model of Fernández (1995), depicted in Fig. 3.3, was constructed for growing pigs and resolved using experimentally determined relationships between model components and P intake and information from a balance study. The model gives explicit consideration to three compartments: (i) gut lumen; (ii) plasma; and (iii) bone (specific pools of P are not nominated), and was constructed to solve single-dose tracer studies. It builds on earlier kinetic models constructed by Aubert and Milhaud (1960) and Aubert *et al.* (1963) for measuring the principal routes of Ca metabolism in man.

Table 3.3. Application of the model of Smith *et al.* (1955) to sheep weighing 36 kg fed a basal diet comprising coast cross hay, cassava flour, soybean meal, urea and a mineral mixture supplemented with 0, 1.5, 3 and 4.5 g/kg DM of monoammonium phosphate (after Dias *et al*., 2009).

		g P per kg feed dry matter			
	Symbol[a]	1.48	2.04	2.57	3.04
Intake (g/day)	F_{10}	1.42	1.92	2.48	3.07
Endogenous secretion into rumen (g/day)	F_{12}	1.05	1.37	1.53	1.91
P turnover time in the rumen (days)		1.42	1.23	1.18	1.04

[a]Symbols according to Fig. 3.2.

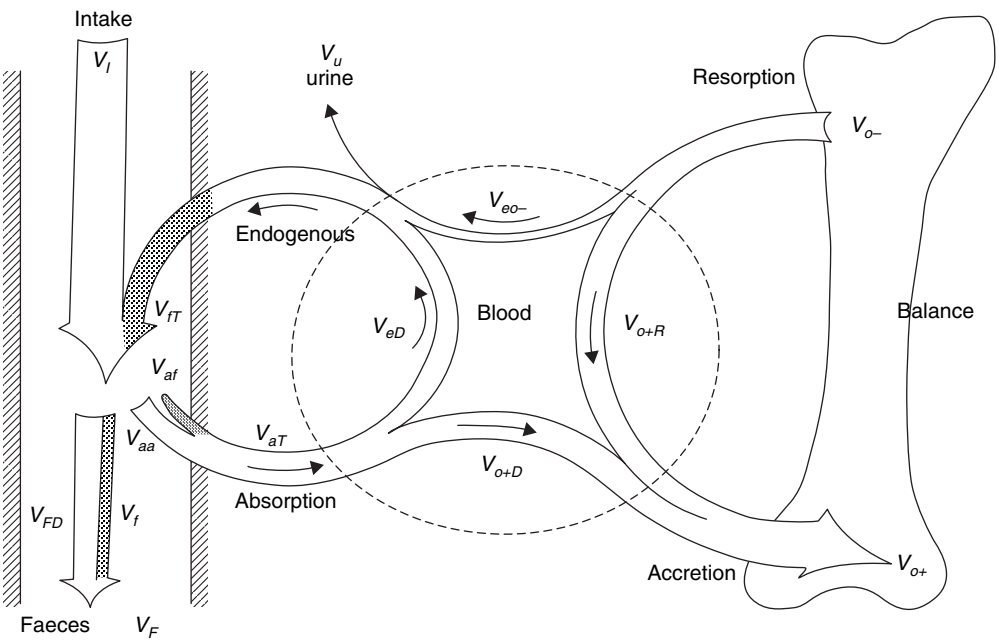

Fig. 3.3. The three-compartment model of Fernández. V_I, daily intake; V_f, excreted P of endogenous origin; V_{FD}, excreted P of dietary origin; V_F, total P excreted in faeces; V_{aa}, absorbed P of dietary origin; V_{af}, absorbed P of endogenous origin; V_{aT}, total absorbed P; V_{o+D}, the contribution of V_{aT} to accretion; V_{o+R}, P from resorption processes recycled via accretion; V_{o+}, total accretion; *Ba*, net deposited P; V_{o-}, total resorbed P; V_{eo-}, resorbed P being excreted or secreted to the gut; V_{eD}, V_{aT} – absorbed P being excreted or secreted to the gut; V_u, P excreted in urine; V_{fT}, total secretion of P into the gut lumen. Adapted from Fernández (1995).

The primary flows are absorption from gut to blood (Eqn 3.4a), blood to gut (Eqn 3.4b), blood to bone (Eqn 3.4c) and bone to blood (Eqn 3.4d) (mathematical notation corresponds to Fernández (1995), as shown in Fig. 3.3, and the equations are as reported by Schulin-Zeuthen *et al.,* 2005):

$$V_{aT} = V_{aa} + V_{af} \tag{3.4a}$$

$$V_{fT} = \frac{V_f}{1 - (V_{aa} / V_I)} \tag{3.4b}$$

$$V_{o+} = \frac{\text{accumulated activity in bone}}{\text{integral of specific radioactivity (SRA) in plasma}} \tag{3.4c}$$

$$V_{o-} = V_{o+} - Ba \tag{3.4d}$$

where the following intermediary equations are applied:

$$V_{aa} = -1.5 + 3.1 \ln V_I$$
$$V_f = V_{aa} - V_I + V_F$$
$$V_{af} = V_{fT} - V_f$$
$$Ba = V_I - V_F - V_u$$

Inputs are P intake (V_I), P excretion in faeces (V_F), P excretion in urine (V_u) and SRA in blood and bone. The following supplementary equations are also included:

$$V_{FD} = V_F - V_f$$
$$V_{o+D} = \frac{V_{o+}}{V_{o-} + V_{aT}} V_{aT}$$
$$V_{o+R} = \frac{V_{o+}}{V_{o-} + V_{aT}} V_{o-}$$
$$V_{eD} = V_{aT} - V_{o+D}$$
$$V_{eo-} = V_{o-} - V_{o+R}$$

Application of the model is illustrated in Table 3.4.

Four-pool Models

Vitti *et al.* (2000)

The model of whole-body phosphorus metabolism proposed by Vitti *et al.* (2000) is shown in Fig. 3.4a. It contains four pools of P: (i) gut lumen; (ii) plasma; (iii) bone; and (iv) soft tissue. The gut, bone and soft tissue pools interchange bidirectionally with the plasma pool, with flows F_{21} and F_{12}, F_{23} and F_{32}, and F_{24} and F_{42}, respectively. Phosphorus entry to the system is via intake (F_{10}), and exit via faeces (F_{01}) and urine (F_{02}). The scheme adopted for the movement of label is shown in Fig. 3.4b. ^{32}P is administered as a single dose (D, μCi) at time

Table 3.4. Application of the model of Fernández (1995) to growing pigs fed a semi-synthetic diet based on barley, sugar, dextrose and cellulose, with different amounts of calcium carbonate and monosodium phosphate to provide different levels of Ca and P for each treatment (after Schulin-Zeuthen *et al.*, 2005).

		Live weight (kg) and level of P intake[b]				
Flow of P (g/day)	Symbol[a]	35L	35M	35H	65L	65M
Intake	V_I	3.00	7.60	11.7	3.70	8.10
Faecal excretion	V_F	1.60	3.20	6.00	2.20	3.80
Urinary excretion	V_u	0.27	1.05	1.85	0.10	0.59
Gut to plasma	V_{aT}	2.79	5.45	6.59	4.91	6.08
Plasma to gut	V_{fT}	1.39	1.05	0.89	3.41	1.78
Plasma to bone	V_{o+}	3.59	3.59	3.87	5.43	5.80
Bone to plasma	V_{o-}	2.46	0.24	0.02	4.03	2.09

[a]Symbols according to Fig. 3.3.
[b]Levels of P intake: low (L), medium (M) and high (H), each with equal Ca:P ratio.

zero, and size of the bone and soft tissues pools and specific activity of all pools are monitored. The scheme assumes there is no re-entry of label from external sources.

Conservation of mass principles can be applied to each pool in Fig. 3.4 to generate differential equations which describe the dynamic behaviour of the system. For unlabelled P, these differential equations are (see Table 3.1 for notation):

$$\frac{dQ_1}{dt} = F_{10} + F_{12} - F_{01} - F_{21} \tag{3.5a}$$

$$\frac{dQ_2}{dt} = F_{21} + F_{23} + F_{24} - F_{02} - F_{12} - F_{32} - F_{42} \tag{3.5b}$$

$$\frac{dQ_3}{dt} = F_{32} - F_{23} \tag{3.5c}$$

$$\frac{dQ_4}{dt} = F_{42} - F_{24} \tag{3.5d}$$

and for ^{32}P:

$$\frac{dq_1}{dt} = s_2F_{12} - s_1(F_{01} + F_{21}) \tag{3.6a}$$

$$\frac{dq_2}{dt} = s_1F_{21} + s_3F_{23} + s_4F_{24} - s_2(F_{02} + F_{12} + F_{32} + F_{42}) \tag{3.6b}$$

$$\frac{dq_3}{dt} = s_2F_{32} - s_3F_{23} \tag{3.6c}$$

$$\frac{dq_4}{dt} = s_2F_{42} - s_4F_{24} \tag{3.6d}$$

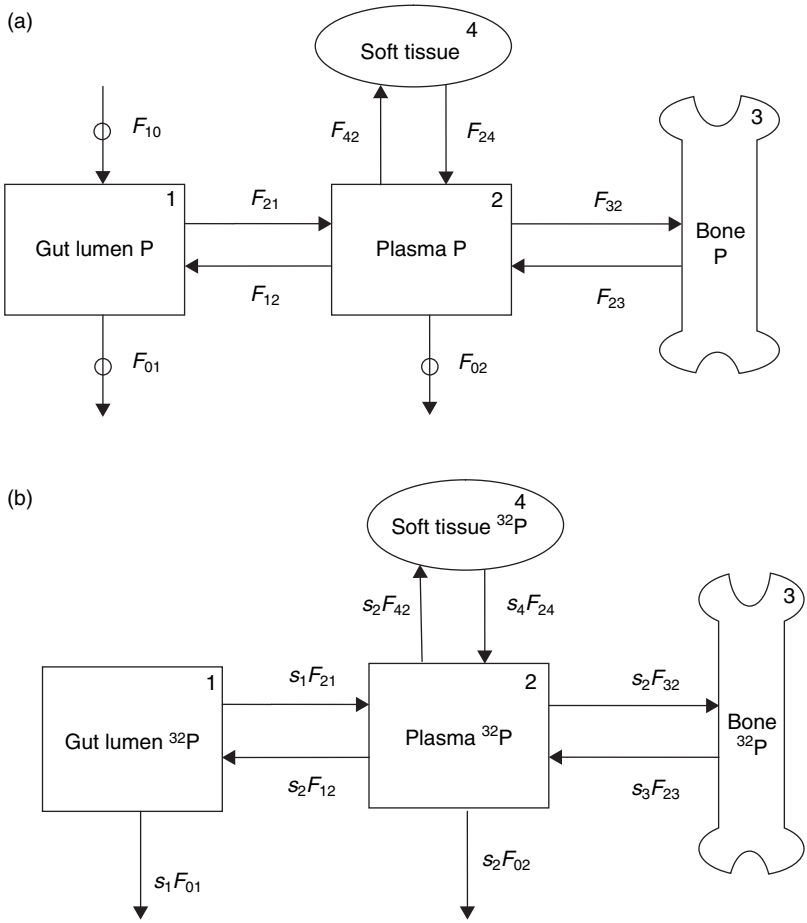

Fig. 3.4. The four-pool model of Vitti *et al.*: (a) unlabelled P and (b) labelled P. F_{ij} represents total P flow to pool *i* from *j*, F_{10} denotes ingestion of P, F_{01} excretion of P in faeces, F_{02} excretion of P in urine. Specific activity of pool *i* is denoted by s_i, and circles denote flows measured experimentally. Adapted from Vitti *et al.* (2000).

Consider the differential coefficient of s_3 with respect to time:

$$\frac{\mathrm{d}s_3}{\mathrm{d}t} = \frac{\mathrm{d}(q_3 Q_3^{-1})}{\mathrm{d}t} = Q_3^{-1}\frac{\mathrm{d}q_3}{\mathrm{d}t} - q_3 Q_3^{-2}\frac{\mathrm{d}Q_3}{\mathrm{d}t} \tag{3.7}$$

Using Eqns 3.5c and 3.6c in Eqn 3.7 gives:

$$\frac{1}{s_3}\frac{\mathrm{d}s_3}{\mathrm{d}t} = \frac{s_2 - s_3}{s_3 Q_3}F_{32} \tag{3.8}$$

Similarly:

$$\frac{1}{s_4}\frac{\mathrm{d}s_4}{\mathrm{d}t} = \frac{s_2 - s_4}{s_4 Q_4} F_{42} \tag{3.9}$$

After N h (typically N is taken to be about a week), it is assumed that pool 1 (gut lumen) is in complete steady state (i.e. both $\mathrm{d}Q_1/\mathrm{d}t$ and $\mathrm{d}q_1/\mathrm{d}t$ are zero) and pool 2 (plasma) is in non-isotopic steady state (i.e. $\mathrm{d}Q_2/\mathrm{d}t$ is zero). It is further assumed that after N h the relative rates of change of specific activity in bone and soft tissue can both be approximated by N^{-1}. Eqns 3.5a, 3.5b, 3.6a, 3.8 and 3.9 now become:

$$F_{10} + F_{12} - F_{01} - F_{21} = 0 \tag{3.10a}$$

$$F_{21} + F_{23} + F_{24} - F_{02} - F_{12} - F_{32} - F_{42} = 0 \tag{3.10b}$$

$$s_2 F_{12} - s_1 (F_{01} + F_{21}) = 0 \tag{3.10c}$$

$$\frac{s_2 - s_3}{s_3 Q_3} F_{32} = \frac{1}{N} \tag{3.10d}$$

$$\frac{s_2 - s_4}{s_4 Q_4} F_{42} = \frac{1}{N} \tag{3.10e}$$

Algebraic manipulation of Eqn 3.10 gives:

$$F_{12} = \frac{s_1}{s_2 - s_1} F_{10} \tag{3.11a}$$

$$F_{21} = F_{10} + F_{12} - F_{01} \tag{3.11b}$$

$$F_{32} = \frac{s_3 Q_3}{N(s_2 - s_3)} \tag{3.11c}$$

$$F_{42} = \frac{s_4 Q_4}{N(s_2 - s_4)} \tag{3.11d}$$

$$\left|F_{23} + F_{24}\right| = F_{02} + F_{12} + F_{32} + F_{42} - F_{21} \tag{3.11e}$$

The combined flow $\left|F_{23} + F_{24}\right|$, which denotes the sum of outflow from pool 3 and outflow from pool 4:

$$\left|F_{23} + F_{24}\right| = F_{23} + F_{24} \tag{3.12}$$

can be partitioned by combining pools 3 and 4. Let s^* denote the specific activity of the combined pool. This is calculated as:

$$s^* = \frac{s_3 Q_3 + s_4 Q_4}{Q_3 + Q_4} \tag{3.13}$$

The outflow of label from the combined pool is the sum of the outflow of label from pool 3 and the outflow of label from pool 4:

$$s^* \times \left|F_{23} + F_{24}\right| = s_3 F_{23} + s_4 F_{24} \tag{3.14}$$

Algebraic manipulation of Eqns 3.12 and 3.14 gives:

$$F_{24} = \frac{s^* - s_3}{s_4 - s_3} \times \left|F_{23} + F_{24}\right| \quad (3.15a)$$

$$F_{23} = \left|F_{23} + F_{24}\right| - F_{24} \quad (3.15b)$$

Regarding the formula for F_{24}, a typographical error appears in Vitti *et al.* (2000). The entity s^* in the denominator of their equation [26] should be s_3, as in Eqn (3.15a) above.

The model is applied by using Eqns 3.11, 3.13 and 3.15 to compute the unknown flows. Application is illustrated in Table 3.5.

Dias *et al.* (2006)

The Dias model is the same as the Vitti model except for some minor amendments. The revised model is shown in Fig. 3.5. Like the original, it contains four P pools: (i) gut lumen; (ii) plasma; (iii) bone; and (iv) soft tissue. The flow F_{10} is now partitioned into P of phytate and non-phytate origins, and F_{01} and F_{21} are partitioned into P of dietary phytate, endogenous and dietary non-phytate origins (see Fig. 3.5). Further, the plasma ^{32}P pool is assumed to decay according to:

$$s_2 = s_2(0)e^{-kt}$$

where k (per h) is a rate constant. Therefore:

$$\frac{ds_2}{dt} = -ks_2(0)e^{-kt}$$

Table 3.5. Application of the model of Vitti *et al.* (2000) to growing goats weighing 20 to 30 kg fed a concentrate mixture and *Brachiaria decumbens* hay supplemented with different levels of dicalcium phosphate.

		Level of P intake		
Flow of P (g/day)	Symbol[a]	Low	Medium	High
Intake	F_{10}	0.42	1.36	3.63
Faecal excretion	F_{01}	0.53	0.94	2.03
Urinary excretion	F_{02}	0.0041	0.0043	0.0057
Gut to plasma	F_{21}	0.16	0.80	2.84
Plasma to gut	F_{12}	0.05	1.22	4.44
Plasma to bone	F_{32}	1.54	2.97	3.39
Bone to plasma	F_{23}	0.63	1.77	2.55
Plasma to tissue	F_{42}	0.14	0.35	0.37
Tissue to plasma	F_{24}	0.93	1.96	2.81

[a]Symbols according to Fig. 3.4.

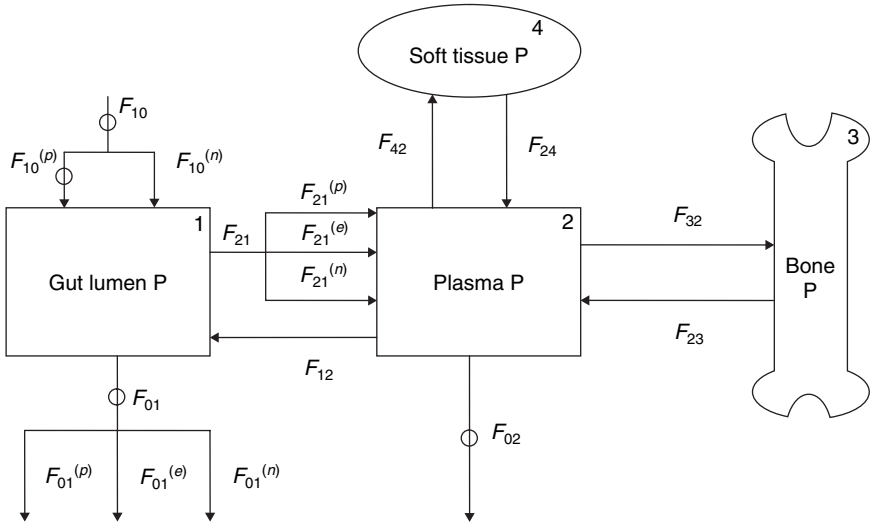

Fig. 3.5. The four-pool scheme of Dias *et al.* showing phytate P. F_{ij} represents total P flow to pool *i* from *j*, F_{10} denotes ingestion of P, F_{01} excretion of P in faeces, F_{02} excretion of P in urine. The flows F_{10}, F_{01} and F_{21} are partitioned as shown, with superscripts (*p*), (*e*) and (*n*) indicating P of dietary phytate, endogenous and dietary non-phytate origin, respectively. Circles denote flows measured experimentally. Adapted from Dias *et al.* (2006).

and the relative rate of change of s_2 is given by:

$$\frac{1}{s_2}\frac{\mathrm{d}s_2}{\mathrm{d}t} = -k$$

The same assumptions apply after N h except that it is now assumed that the relative rates of change of specific activity in plasma, bone and soft tissue reach the same magnitude k, rather than $s_3^{-1}\mathrm{d}s_3/\mathrm{d}t = s_4^{-1}\mathrm{d}s_4/\mathrm{d}t = N^{-1}$ previously. Eqns 3.10d and 3.10e now become:

$$\frac{s_2 - s_3}{s_3 Q_3} F_{32} = k$$

$$\frac{s_2 - s_4}{s_4 Q_4} F_{42} = k$$

giving:

$$F_{32} = \frac{k s_3 Q_3}{s_2 - s_3} \tag{3.16a}$$

$$F_{42} = \frac{k s_4 Q_4}{s_2 - s_4} \tag{3.16b}$$

As ingested phytate P ($F_{10}^{(p)}$) is measured, P excretion in faeces (F_{01}) and P absorption (F_{21}) can be partitioned between P sources using the following equations:

$$F_{01}^{(p)} = z_p F_{01} \quad (3.17a)$$

$$F_{01}^{(e)} = z_e F_{01} \quad (3.17b)$$

$$F_{01}^{(n)} = F_{01} - F_{01}^{(p)} - F_{01}^{(e)} \quad (3.17c)$$

$$F_{21}^{(p)} = z_p F_{21} \quad (3.17d)$$

$$F_{21}^{(e)} = z_e F_{21} \quad (3.17e)$$

$$F_{21}^{(n)} = F_{21} - F_{21}^{(p)} - F_{21}^{(e)} \quad (3.17f)$$

where:

$$z_p = \frac{F_{10}^{(p)}}{F_{10} + F_{12}} \quad (3.18a)$$

$$z_e = \frac{F_{12}}{F_{10} + F_{12}} \quad (3.18b)$$

Superscript (*p*) denotes P of dietary phytate origin, (*e*) endogenous P, and (*n*) P of dietary non-phytate origin.

The model is applied by using Eqns 3.11 (with Eqns 3.16 replacing 3.11c, d), 3.13, 3.15, 3.17 and 3.18 to compute the unknown flows. Application is illustrated in Table 3.6.

Table 3.6. Application of the model of Dias *et al.* (2006) to sheep weighing 32 kg fed a basal diet containing hydrolysed sugarcane bagasse, maize, soybean meal, a mineral mixture and urea, and supplemented with a different source of Ca in each treatment: limestone, citrus pulp or oyster shell meal.

Flow of P	Symbol[a]	Amount[b] (g/day)
Intake	F_{10}	3.19
Phytate	$F_{10}^{(p)}$	1.29
Non-phytate	$F_{10}^{(n)}$	1.89
Faeces	F_{01}	4.47
Phytate	$F_{01}^{(p)}$	0.68
Non-phytate	$F_{01}^{(n)} + F_{01}^{(e)}$	3.79
Gut to plasma	F_{21}	3.08
Plasma to gut	F_{12}	4.37
Plasma to bone	F_{32}	5.70
Bone to plasma	F_{23}	8.75
Plasma to tissue	F_{42}	2.72
Tissue to plasma	F_{24}	1.07

[a]Symbols according to Fig. 3.5.
[b]Values correspond to mean of all treatments.

A Six-pool Model

This model of whole-body phosphorus metabolism is an extension of the one proposed by Vitti *et al.* (2000) to six pools for use in ruminants. It is illustrated in Fig. 3.6. The six pools of P are: (i) rumen; (ii) lower gastrointestinal tract; (iii) saliva; (iv) plasma; (v) bone; and (vi) soft tissue. Phosphorus entry to the system is via intake (F_{10}), and exit via faeces (F_{02}) and urine (F_{04}). ^{32}P is administered as a single dose (D, μCi) into systemic blood at time zero, and size of the bone and soft tissues pools and specific activity of all pools are monitored. The scheme assumes there is no re-entry of label from external sources.

Conservation of mass principles can be applied to each pool in Fig. 3.6 to generate differential equations. For unlabelled P:

$$\frac{dQ_1}{dt} = F_{10} + F_{13} - F_{21} \tag{3.19a}$$

$$\frac{dQ_2}{dt} = F_{21} + F_{24} - F_{02} - F_{42} \tag{3.19b}$$

$$\frac{dQ_3}{dt} = F_{34} - F_{13} \tag{3.19c}$$

$$\frac{dQ_4}{dt} = F_{42} + F_{45} + F_{46} - F_{04} - F_{24} - F_{34} - F_{54} - F_{64} \tag{3.19d}$$

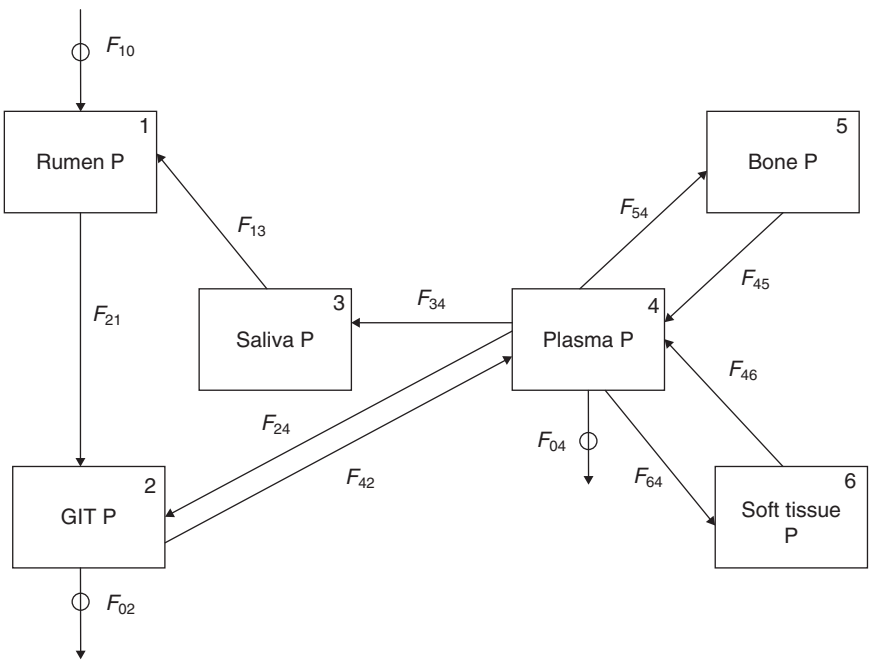

Fig. 3.6. A six-pool model. F_{ij} represents total P flow to pool i from j, F_{10} denotes ingestion of P, F_{02} excretion of P in faeces, F_{04} excretion of P in urine. Circles denote flows measured experimentally.

$$\frac{dQ_5}{dt} = F_{54} - F_{45} \tag{3.19e}$$

$$\frac{dQ_6}{dt} = F_{64} - F_{46} \tag{3.19f}$$

and for ^{32}P:

$$\frac{dq_1}{dt} = s_3F_{13} - s_1F_{21} \tag{3.20a}$$

$$\frac{dq_2}{dt} = s_1F_{21} + s_4F_{24} - s_2(F_{02} + F_{42}) \tag{3.20b}$$

$$\frac{dq_3}{dt} = s_4F_{34} - s_3F_{13} \tag{3.20c}$$

$$\frac{dq_4}{dt} = s_2F_{42} + s_5F_{45} + s_6F_{46} - s_4(F_{04} + F_{24} + F_{34} + F_{54} + F_{64}) \tag{3.20d}$$

$$\frac{dq_5}{dt} = s_4F_{54} - s_5F_{45} \tag{3.20e}$$

$$\frac{dq_6}{dt} = s_4F_{64} - s_6F_{46} \tag{3.20f}$$

Let:

$$s_4 = s_4(0)e^{-kt}$$

where k (per h) is a rate constant. Then:

$$\frac{ds_4}{dt} = -ks_4(0)e^{-kt}$$

and:

$$\frac{1}{s_4}\frac{ds_4}{dt} = -k$$

Consider:

$$\frac{ds_5}{dt} = \frac{d(q_5Q_5^{-1})}{dt} = Q_5^{-1}\frac{dq_5}{dt} - q_5Q_5^{-2}\frac{dQ_5}{dt} \tag{3.21}$$

Using Eqns 3.19*e* and 3.20*e* in Eqn 3.21 gives:

$$\frac{1}{s_5}\frac{ds_5}{dt} = \frac{s_4 - s_5}{s_5Q_5}F_{54} \tag{3.22}$$

Similarly:

$$\frac{1}{s_6}\frac{ds_6}{dt} = \frac{s_4 - s_6}{s_6Q_6}F_{64} \tag{3.23}$$

At time N h after dosing (N is assumed to be about a week), assume that both gut pools reach complete steady state, i.e.:

$$\frac{dQ_1}{dt} = F_{10} + F_{13} - F_{21} = 0 \tag{3.24a}$$

$$\frac{dQ_2}{dt} = F_{21} + F_{24} - F_{02} - F_{42} = 0 \tag{3.24b}$$

$$\frac{dq_1}{dt} = s_3F_{13} - s_1F_{21} = 0 \tag{3.24c}$$

$$\frac{dq_2}{dt} = s_1F_{21} + s_4F_{24} - s_2(F_{02} + F_{42}) = 0 \tag{3.24d}$$

and that the saliva and plasma pools reach non-isotopic steady state, i.e.:

$$\frac{dQ_3}{dt} = F_{34} - F_{13} = 0 \tag{3.25a}$$

$$\frac{dQ_4}{dt} = F_{42} + F_{45} + F_{46} - F_{04} - F_{24} - F_{34} - F_{54} - F_{64} = 0 \tag{3.25b}$$

Solving Eqns 3.24a and 3.24c:

$$F_{13} = \frac{s_1F_{10}}{s_3 - s_1} \tag{3.26}$$

Eqn 3.24a then gives:

$$F_{21} = F_{10} + F_{13} \tag{3.27}$$

Solving Eqns 3.24b and 3.24d:

$$F_{24} = \frac{s_1 - s_2}{s_2 - s_4}F_{21} \tag{3.28}$$

Eqn 3.24b and 3.25a then yield, respectively:

$$F_{42} = F_{21} + F_{24} - F_{02} \tag{3.29a}$$

$$F_{34} = F_{13} \tag{3.29b}$$

Assume further that at time N the relative rates of change of specific activity in plasma, bone and soft tissue have the same magnitude, i.e.:

$$\frac{1}{s_5}\frac{ds_5}{dt} = \frac{1}{s_6}\frac{ds_6}{dt} = k$$

Eqns 3.22 and 3.23 then yield, respectively:

$$F_{54} = \frac{ks_5Q_5}{s_4 - s_5} \tag{3.30a}$$

$$F_{64} = \frac{ks_6Q_6}{s_4 - s_6} \tag{3.30b}$$

Alternatively, at time N approximate ds_5/dt in Eqn 3.22 by s_5/N and ds_6/dt in Eqn 3.23 by s_6/N. Eqns 3.22 and 3.23 then give, respectively:

$$F_{54} = \frac{s_5 Q_5}{(s_4 - s_5)N} \tag{3.31a}$$

$$F_{64} = \frac{s_6 Q_6}{(s_4 - s_6)N} \tag{3.31b}$$

Eqn 3.25b now yields:

$$\left|F_{45} + F_{46}\right| = F_{04} + F_{24} + F_{34} + F_{54} + F_{64} - F_{42} \tag{3.32}$$

The combined flow $\left|F_{45} + F_{46}\right|$, which denotes the sum of outflow from pool 5 and pool 6, i.e.:

$$\left|F_{45} + F_{46}\right| = F_{45} + F_{46} \tag{3.33}$$

can be uncoupled by combining pools 5 and 6. Let s^* denote the specific activity of the combined pool. This is calculated as:

$$s^* = \frac{s_5 Q_5 + s_6 Q_6}{Q_5 + Q_6} \tag{3.34}$$

The outflow of label from the combined pool is the sum of the outflow of label from pool 5 and the outflow of label from pool 6:

$$s^* \times \left|F_{45} + F_{46}\right| = s_5 F_{45} + s_6 F_{46} \tag{3.35}$$

Algebraic manipulation of Eqns 3.33 and 3.35 gives:

$$F_{46} = \frac{s^* - s_5}{s_6 - s_5} \times \left|F_{45} + F_{46}\right| \tag{3.36a}$$

$$F_{45} = \left|F_{45} + F_{46}\right| - F_{46} \tag{3.36b}$$

The model can be applied by computing the flows using Eqns 3.26–3.30, 3.32, 3.34 and 3.36. A second solution to the model can be found using Eqn 3.31 instead of 3.30 to compute the flows F_{54} and F_{64}. If ingested phytate P ($F_{10}^{(p)}$) is measured, P excretion in faeces (F_{02}) and P absorption (F_{42}) can be partitioned between P sources using the following equations:

$$F_{02}^{(p)} = z_p F_{02}$$
$$F_{02}^{(e)} = z_e F_{02}$$
$$F_{02}^{(n)} = F_{02} - F_{02}^{(p)} - F_{02}^{(e)}$$
$$F_{42}^{(p)} = z_p F_{42}$$
$$F_{42}^{(e)} = z_e F_{42}$$
$$F_{42}^{(n)} = F_{42} - F_{42}^{(p)} - F_{42}^{(e)}$$

where:

$$z_p = \frac{F_{10}^{(p)}}{F_{10} + F_{13} + F_{24}}$$

$$z_e = \frac{F_{13} + F_{24}}{F_{10} + F_{13} + F_{24}}$$

Superscript (*p*) denotes P of dietary phytate origin, (*e*) endogenous P and (*n*) P of dietary non-phytate origin. Application is illustrated in Table 3.7.

An Eight-pool Model

Schneider *et al.* (1987)

The scheme reported by Schneider *et al.* (1987) is shown in Fig. 3.7. It contains eight exchangeable pools of P: (i) blood; (ii) soft tissue; (iii) bone; (iv) rumen; (v) abomasum and upper small intestine; (vi) lower small intestine; (vii) colon and caecum; and (viii) kidney, with a delay between upper and lower small intestine. It was used to analyse kinetic data from an experiment using chaff-fed and liquid-fed sheep dosed with labelled P and developed using SAAM, the simulation, analysis and modelling software (SAAM Institute, 1997; Barrett *et al.*, 1998). Calculation of pool sizes and rates of flow between pools involves the assumption of steady state during the experiment. Parameters used to describe the model and used in the SAAM program are L_{ij}, the fraction of tracer flowing into pool *i* from pool *j* per minute, M_i, the quantity of P (g) contained in pool *i* during

Table 3.7. Application of the six-pool model to growing sheep weighing 33 kg, fed a basal diet comprising coast cross hay, cassava flour, soybean meal, urea and a mineral mixture supplemented with phosphorus (unpublished data).

		g P per kg feed dry matter			
Flow of P (g/day)	Symbol[a]	1.4	3.1	4.9	6.5
Intake	F_{10}	1.4	3.5	5.6	7.5
Faecal excretion	F_{02}	1.1	3.2	4.4	6.5
Urinary excretion	F_{04}	0.012	0.010	0.071	0.355
Rumen to gut	F_{21}	2.61	5.57	8.48	11.73
Saliva to rumen	F_{13}	1.15	2.04	2.90	4.26
Plasma to saliva	F_{34}	1.15	2.04	2.90	4.26
Plasma to gut	F_{24}	0.96	2.85	3.98	1.35
Gut to plasma	F_{42}	2.47	5.24	8.07	6.53
Plasma to bone	F_{54}	12.50	14.26	16.39	16.18
Bone to plasma	F_{45}	12.31	14.07	16.13	16.02
Plasma to tissue	F_{64}	2.66	3.26	4.23	4.12
Tissue to plasma	F_{46}	2.50	3.11	3.38	3.71

[a]Symbols according to Fig. 3.6.

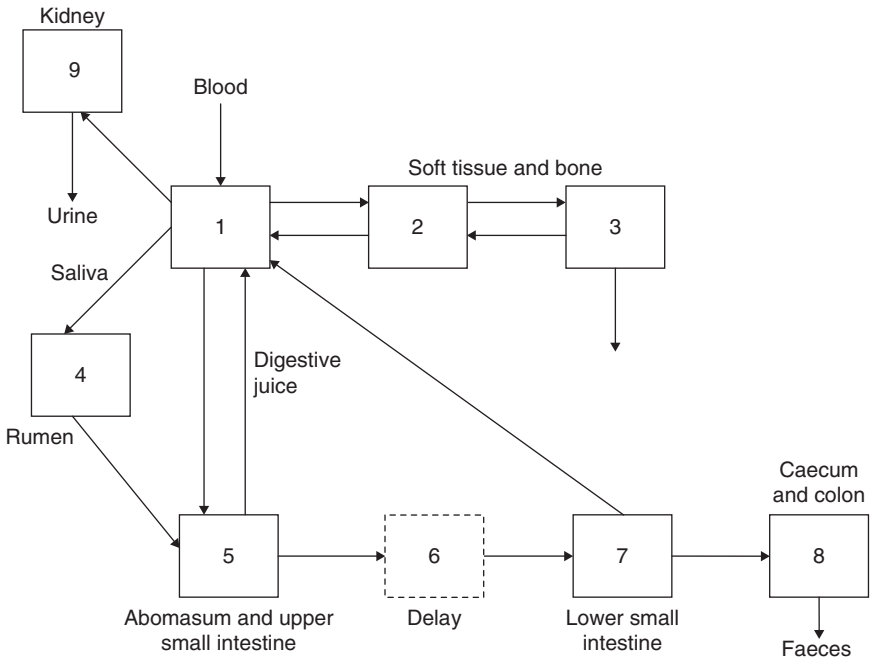

Fig. 3.7. The eight-pool scheme of Schneider *et al.* constructed using SAAM software. Solid-line boxes represent exchangeable pools of P and the dashed-lined box represents a delay. Adapted from Schneider *et al.* (1987).

steady-state conditions, and R_{ij}, which is equal to $L_{ij} \times M_i$ and is the steady-state flow of P (g/day) moving into pool *i* from *j*. Application is illustrated in Table 3.8. Other applications of SAAM to study P kinetics in sheep include Grace (1981) and Schneider *et al.* (1982, 1985), who report a five-pool model and an eight-pool model with a delay, respectively.

Discussion

The primary kinetic models used for studying P metabolism in ruminants and monogastrics have been presented in this chapter. The work of Lofgreen and Kleiber (1953), where isotope dilution was applied to determine endogenous P in faeces, represents an important early contribution. Based on previous studies with lambs, these authors assumed that the amount of ^{32}P entering the total gastrointestinal tract (GIT) one day is excreted in faeces 24 h later. Therefore, endogenous P flow was determined after measuring ^{32}P in blood at 0 h and faeces at 24 h. Furthermore, the authors used the values of endogenous faecal P, daily P intake and daily P excretion in faeces to obtain true digestibility of P in lucerne hay.

Smith *et al.* (1955) used these basic concepts to develop a simple model representing P inflows to the rumen from the diet and plasma, and outflows to

Table 3.8. Model flows and proposed physiological equivalents for sheep weighing 38–42 kg, fed 700 g/day lucerne and oat chaff (i.e. chopped hay) containing 3.75 g calcium and 1.4 g phosphorus (Schneider *et al.*, 1987).

Symbol[a]	Amount, g P/day	Proposed physiological equivalent
R_{12}	12.82	Flows between plasma, extracellular fluid, tissue and bone
R_{21}	13.18	
R_{23}	1.10	
R_{32}	1.46	
R_{03}	0.36	Flow to unexchangeable tissue and bone
R_{51}	0.27	Digestive juice
R_{41}	6.70	Saliva
R_{54}	7.88	Rumen outflow
R_{15}	5.05	Absorption from upper intestine
R_{65}	3.10	Flow from upper to lower intestine
R_{17}	2.29	Absorption from lower intestine
$R_{87} + R_{08}$	0.82	Flow to caecum, colon and faeces
$R_{91} + R_{09}$	0.004	Flow to kidney and urine
U_4	1.18	Dietary intake

[a]Symbols according to text and Fig. 3.7: first subscript represents flow destination and second flow origin.

plasma and the lower GIT. Also, samples from segments of the GIT were collected for radioactivity and P measurements, and values related to the rumen were further used to resolve the model.

Vitti *et al.* (2000) presented a model based on isotope dilution to study P movement between the following compartments: soft tissues, bone, plasma and GIT. In building the model, no re-entry of label from external sources was assumed and conservation of mass principles was applied to each pool to generate differential equations that account for P exchanges between pools. This model has been shown to be useful in understanding P metabolism in both ruminants and monogastrics. For example, the Vitti model demonstrated major differences between endogenous P flows in monogastrics (Schulin-Zeuthen *et al.*, 2005) and ruminants (Vitti *et al.*, 2000), affirming that endogenous P excretion is considerably higher in ruminants than in monogastrics. The Vitti model was also shown to be useful in studying the effect of different Ca sources on Ca metabolism in ruminants (Dias *et al.*, 2008).

The Vitti model was revised by Dias *et al.* (2006). Data on phytate ingestion and excretion were used to add three sub-flows from GIT to plasma representing endogenous P, dietary phytate P and dietary non-phytate P. Phosphorus excreted in faeces was partitioned similarly. The pools represented remained the same as in the original model. Two pools were subsequently added to the Vitti–Dias model to represent explicitly the rumen and saliva compartments, as described in this chapter. Consequent upon this model development, samples of saliva and rumen content were collected by Vitti and co-workers over 3 days in addition to

the usual daily plasma and faeces collections for radioactive and non-radioactive P measurements. The P flows between saliva and the rumen, saliva and plasma, saliva and lower GIT and the rumen and lower GIT are calculated using these saliva and rumen P data. Since saliva and the rumen contain considerable amounts of P, this model gives a more detailed and accurate representation of P metabolism in the ruminant.

Schneider *et al.* (1987) undertook compartmental analysis using the software SAAM (SAAM Institute, 1997; Barrett *et al.*, 1998). The authors used tracer data from an experiment where sheep fed a chaff diet and a liquid diet were given ^{32}P and ^{33}P intravenously and gastrointestinally, and blood, rumen, abomasum, urine and faeces samples were collected. Initially, three- and four-pool models were considered. Pools representing the GIT and flow of P within and from the tract were added to fit the blood disappearance curve obtained after abomasal injection of tracer. The resultant model contained eight pools. Phosphorus recycling from saliva was represented in the model, and likewise endogenous secretion of P (coming mainly from digestive juice) and P outflow from the rumen. The authors suggested a link between salivary gland and kidney based on P flux to kidney and urine and P salivation when sheep were transferred from the chaff to the liquid diet.

The SAAM software was also used earlier by Schneider *et al.* (1985) to determine Ca and P absorption in young sheep. The assumptions made in building a model with SAAM are: (i) the kinetics of the system is linear; (ii) the tracer mixes completely with tracee in the pools represented; and (iii) the metabolism of the animal is in steady state. The model for Ca has two absorption pools and a delay between them, while the model for P has one primary absorption pool, three secondary ones and a delay between the primary and secondary pools.

Fernández (1995) proposed a model to study Ca and P metabolism in growing pigs which utilizes absorption, balance and tracer data. The model comprises three compartments (GIT, bone and plasma) and considers flows such as absorption, excretion, mineral accretion and resorption from bone. Some of the model assumptions are that endogenous Ca is absorbed to the same extent as dietary Ca, and Ca entering the central pool is readily and thoroughly mixed. Since most Ca is found in bone, Ca balance was assumed to be the difference between Ca accretion and resorption in bone. Regarding P balance, a different approach was followed because, though a considerable portion of P is present in bone, a substantial portion is found in soft tissues. Therefore P retention was distributed between bone and soft tissues in the same proportion as found in body composition analysis.

Kinetic models have also been used to study Ca metabolism alone, mostly in humans. Given the close relationship between Ca and P metabolism, these models are of relevance to our discussion here. Short descriptions of some of the models published now follow.

Aubert and Milhaud (1960) proposed a method to measure the main routes of Ca exchange in humans. The method was based on a mathematical analysis of the curve representing the decrease of Ca specific activity in serum. The model has three pools: GIT, plasma and bone plus soft tissues. To determine total Ca loss from the central pool (plasma), the decay curve was separated into two parts: one representing rapid exchanges corresponding to the period covering

the first 48 h after injection of the radioisotope, and the second representing slow exchanges corresponding to the period 48–168 h after injection. Determinations of specific activities of Ca in urine and stool, together with the amounts of Ca ingested and excreted, were used to resolve the model.

Birge *et al.* (1969) developed a mathematical model of Ca absorption in humans in which deconvolution was used to derive a function describing the rate of entry of Ca into plasma after oral dosing. A mechanistic model was then constructed to describe the characteristics of the derived input function. No absorption occurs from the first compartment, which is followed by two segments representing separated delay sections and then a compartment through which material passes before it enters the circulation.

Heideger and Ferguson (1985) proposed a model to study Ca absorption from the intestinal lumen. There are three pools for Ca exchange in this model: (i) the gut; (ii) the blood and rapid-exchange pool; and (iii) the slow-exchange pool and losses to environment. This compartmentalization was used to derive a kinetic equation for fitting to plasma specific activities.

Sen and Mohr (1990) used a different scheme to describe Ca distribution in the human body. They proposed a more general kinetic model containing four pools representing: (i) dose not yet absorbed in the GIT; (ii) Ca circulating in extracellular fluid (ECF); (iii) Ca in storage; and (iv) Ca lost to the body from ECF. Dietary Ca enters pool 1, and a fraction is absorbed from pool 2, where it mixes with existing Ca and may be dispatched to pool 3 for bone mineralization. For model solution it is assumed that Ca lost from ECF contains dose:Ca in the same ratio as dose:Ca present in the ECF pool.

The models described herein aim to enhance our knowledge of P and Ca metabolism in ruminants and monogastrics. Furthermore, the information provided by these models can be useful in developing strategies to improve usage of these minerals, consequently reducing their excretion to the environment.

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4 Phosphorus and Calcium Utilization in Ruminants Using Isotope Dilution Technique

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Introduction

Phosphorus (P) is an essential nutrient involved not only in bone growth and productivity, but also with most of the metabolic processes of the body. Phosphorus is a component of amino acids, proteins, lipids and nucleic acids (Ternouth, 1990). On average, 80% of P in the body is found in bones as hydroxyapatite (McDowell, 1992). In normal conditions, the ash content of P is about 16–17%. During chronic or prolonged deficiency, P is mobilized from bone to maintain normal concentration in blood. Roughly, 17–20% of P is distributed in soft tissues and body fluids (Georgievskii, 1982), mainly as organic P, participating in the transport and metabolism of fats, and the absorption and utilization of carbohydrates and proteins. P is involved in energy metabolism as part of ATP and is also a key component of many coenzymes (Ternouth, 1990).

Total blood contains 350–450 mg P/l, mostly present in cells. When P supply in the diet is adequate, P in plasma, mainly as inorganic P, is between 40 and 90 mg/l. When there is a condition of P depletion, values decline rapidly to levels lower than 40 mg/l (Underwood and Suttle, 1999; Valk *et al*., 2000), but they are rapidly restored by P repletion from bone. Plasma is not a reliable indicator of P status because of the quick compensatory reactions on P repletion (Bortolussi *et al*., 1988; Valk *et al*., 2000). Some studies showed that there is a relationship between P in plasma and deficient to moderate P intake (Scott *et al*., 1985; Ternouth and Sevilla, 1990). In contrast, other studies showed no relationship between these parameters (Louvandini and Vitti, 1994; Louvandini, 1995).

Rumen P content consists of feed and salivary P. The P concentration is between 200 and 600 mg/l (Witt and Owes, 1983), 50–70% of which has

 Phosphorus and Calcium Utilization and Requirements in Farm Animals (D.M.S.S. Vitti and E. Kebreab)

endogenous origin from saliva. Salivary secretions of P constitute about 80% of the endogenous P recycled to the gastrointestinal tract. Salivary P contributes to maintaining adequate pH (6–7) to permit microbial activity and is also a component of microbial cells as part of nucleic acids and phospholipids. Microbial cells have 20–60 g P/kg dry matter (Hungate, 1966). Phosphorus is essential for microbial growth and *in vitro* experiments suggest that 100 mg P/l is an adequate level to maintain microbial growth and cellulolytic activity (Durand and Kawashima, 1980). The daily secretion rate of salivary P is 5–10 g in sheep and 30–60 g in cows (Breves and Schröder, 1991) and depends on variables such as dry matter intake, P intake and the fibre content of the diet (CSIRO, 1990; Valk *et al*., 2000).

The major routes of excretion of P are kidney and gut. In monogastrics the kidney is of greater importance but, normally, urinary P is an alternative P excretion pathway in ruminants (Underwood and Suttle, 1999; Bravo *et al*., 2003b). Ruminants excrete less than 2 mg P/kg live weight (LW) in urine under normal conditions (Ternouth, 1990). High urinary P excretion can occur with abnormally high efficiency of P absorption, when plasma P concentration exceeds the renal threshold (usually between 6 and 9 mg/dl) (Challa and Braithwaite, 1988a, b, c). Urinary P excretion might also be genetically predetermined (Field and Woolliams, 1984). Nevertheless, despite its high variability, urinary P excretion contributes to P homeostasis in ruminants (Bravo *et al*., 2003b).

Ruminants excrete P mainly in faeces, which is composed of unabsorbed dietary P and endogenous P (from saliva, digestive juices, intestinal cells). Total P excretion in faeces depends largely on P intake (Ternouth, 1989; Louvandini and Vitti, 2007). Endogenous P levels may present the highest degree of variation between different physiological conditions (McDowell, 1992; Valk *et al*., 2000). The ratio of endogenous faecal P to total faecal P is highly variable, from 0.12 to 0.95 (Bravo and Meschy, 2003), depending on the age, diet and physiological conditions of the animals. Endogenous P loss can also be related to P intake (Louvandini and Vitti, 2007).

Dietary P is present as inorganic phosphates and organic P mainly as phytate, phospholipids and phosphoproteins. Inorganic P is normally solubilized by acid gastric juices in the small intestine. Phytate P is poorly digested in the small intestine but it can be released by action of microbial enzymes in the rumen (Bravo and Meschy, 2003). In monogastrics a small part of phytate P is hydrolysed in the stomach by vegetable phytase (Georgievskii, 1982). Addition of phytase to poultry and swine diets releases phytate P, thereby reducing the need to supplement diets with inorganic P and mitigating P excretion (Nahm, 2004).

The major site of P absorption is the small intestine (Reinhardt *et al*., 1988; Breves and Schröder, 1991). According to some workers (Yano *et al*., 1991) there is a negligible absorption of P in the reticulum-rumen, but this contradicts other studies that show appreciable P absorption through the rumen wall (Breves *et al*., 1988). Phosphorus absorption also occurs in the omasum (Engelhardt and Hauffe, 1975), but this is negligible in relation to the main site of absorption, especially for small ruminants, due to the size of the organ. Absorption in single-stomach

species also occurs mainly in the small intestine, and P transport is stimulated by vitamin D (Jungbluth and Binswanger, 1989).

Generally P absorption combines a saturated active mechanism and a passive diffusion, which predominates when P concentration in the intestinal lumen is high (Breves and Schröder, 1991). The amount of P absorption depends on the source, intake level, Ca:P ratio, intestinal pH, age and dietary levels of other minerals (Braithwaite, 1984; Scott *et al*., 1984a). Individual variation in P absorption may occur due to genetic differences and it is reflected in P in plasma and urine (Field *et al*., 1984).

Many studies have shown that there is a linear relationship between absorbed P and P intake (Braithwaite, 1984; Coates and Ternouth, 1992; Louvandini and Vitti, 1994; Vitti *et al*., 2000). The efficiency of P absorption increases with increased P supply; however, at high levels of P intake, a curvilinear relationship was observed between P intake and efficiency of absorption (Challa *et al*., 1989). In situations where there is high P demand, such as at the onset of lactation, P absorption efficiency is high, but it decreases when P is supplied in excess of requirements (Braithwaite, 1985; Challa *et al*., 1989). At P intakes between 20 and 80 mg/kg LW/day, efficiency of P absorption in sheep is high (0.74). Louvandini (1995) did not observe a decreased efficiency of absorption with increased P intake in sheep and reported a value of 0.73 at an excessive P intake level of 114 mg/kg LW/day.

In this chapter the results of several experiments with sheep, goats and bovines are described (Vitti *et al*., 2000, 2008; Dias *et al*., 2007). These experiments were performed using the isotope dilution technique described in Chapter 2 and compared with the chemical balance technique. This technique allows the determination of the endogenous faecal losses of P, Ca and other minerals and the determination of the true absorption of the mineral. The knowledge of endogenous P is especially important in the study of P for ruminant nutrition, since this obligatory loss is used to determine mineral requirements.

Phosphorus Utilization in Cattle

In cattle, the most common mineral deficiency present in different regions worldwide is phosphorus. As this mineral has a complex metabolism and different levels of deficiency may occur, especially in animals reared in pasture, national recommendations for requirements show wide variations (INRA, 1988; CSIRO, 1990; AFRC, 1991; NRC, 2001). Few studies have been carried out to understand the mechanisms involved in deficiency and supplementation better, especially those associated with different production situations and bone metabolism (Karn, 2001). Despite revisions of P requirement by different national organizations, controversy still persists regarding P requirements for cattle. The technique of isotopic dilution using ^{32}P has been shown to be an important tool in clarifying the metabolism of this mineral in ruminants with greater accuracy. Nevertheless, there has been a reduction in the number of studies using radioisotopes in cattle due to their size and care required in using this method of analysis.

Phosphorus requirement

Apparent P requirements vary for a variety of reasons, such as differences in cattle breed, P availability in the feed, management (pen fed or grazing), possible interactions between nutrients and the effects of disease and parasitism (Underwood and Suttle, 1999). Using the isotopic dilution technique, Challa and Braithwaite (1988c) showed that the endogenous loss in cattle is not constant and is related to the amount of P consumed, with this loss representing an important mechanism in the homeostasis control of P. Studies on endogenous loss are important for understanding P metabolism better and calculating requirements. Once the minimum P requirement of the animal is known, the amount of P needed to meet production requirements can be calculated with greater precision. With this in mind, Silva Filho *et al*. (2000) carried out ^{32}P studies with Nelore calves weighing 190 kg and determined the minimum endogenous loss as 5.72 mg/kg live weight (LW)/day:

$$\text{Endogenous P} = 0.27\ P_{intake} + 5.72\ (r^2 = 0.60,\ n = 18,\ P < 0.01) \quad (4.1)$$

This value is lower than that determined by Challa *et al*. (1989), which was 8.6 mg/kg LW/day in Friesian bull calves (100–150 kg LW). The Agricultural Research Council (ARC, 1980) stipulated a value of 10 mg/kg LW/day. Ternouth *et al*. (1996) identified an obligatory endogenous P loss for growing cattle ranging from 9 to 17 mg P/kg LW, which was related to dry matter (DM) intake in the range of 10–25 g/kg LW. Other parameters that may be related to P metabolism are given in Table 4.1.

Using the mean absorption coefficient of 0.65 (Silva Filho *et al*., 2000), the daily dietary requirement for maintenance in Nelore cattle would be 8.84 mg/kg LW/day. Pires *et al*. (1993) recommended 12.2 mg/kg LW/day total P for Nelore cattle weighing about 200 kg. The authors used the values of endogenous faecal loss and availability for their requirement calculations from ARC (1980), which employed the comparative slaughter technique.

Another key point in the calculations of P requirements has been biological availability, which has also been a source of disagreement between studies. It has

Table 4.1. Effect of dietary P concentrations on P metabolism in *Bos indicus,* Nelore (Silva Filho *et al*., 2000).

	Treatments[a]			
Variables	0.12	0.24	0.36	Regression
Live weight (kg)	193	197	182	
P intake (mg/kg LW)	26	44	69	
P plasma (mg/100 ml)	6	8	9	$P_{plasma} = 0.06\ P_{intake} + 4.9$ ($r^2 = 0.60$, $P < 0.01$)
P in faeces (mg/kg LW)	23	32	44	$P_{faeces} = 0.53\ P_{intake} + 5.72$ ($r^2 = 0.78$, $P < 0.01$)
P availability (coefficient)	0.55	0.70	0.69	Not significant

[a]Proportion of P in diet as dicalcium phosphate (%).

been proposed that this variable is only related to type of feed, ignoring the animal component (age, sex, breed, physiological state). In this context there are variations between the suggested recommendations. For example the National Research Council (NRC, 2001) uses a coefficient of 0.65 for forages and 0.70 for concentrates in cattle. In the revision carried out by Bravo *et al*. (2003a), the value for ruminants was 0.72. Silva Filho *et al*. (2000) determined the mean coefficient as 0.65 for calves that received dicalcium phosphate supplementation. In addition to type of feed and animal category, the source of P in the diet has an important role in the absorption of this element, which should be taken into consideration in calculations of availability.

Alternative sources of phosphorus

With the increase in prices of inorganic sources of P for herd supplementation, the pressure to use alternative sources has been increasing. Using isotopic dilution, Silva Filho *et al*. (1992) studied monoammonium phosphate, superphosphate and urea-phosphate and compared them with dicalcium phosphate. They reported true absorption coefficients of 0.58, 0.65, 0.62 and 0.68, respectively. Vitti *et al*. (2001) compared the kinetics of dicalcium phosphate, Patos de Minas and Tapira rock phosphates in cattle and the main results are shown in Table 4.2.

The flow of P from the gastrointestinal tract (GIT) to blood and vice versa was greater in cattle fed diets with dicalcium phosphate while no significant differences were found between the treatments with Patos de Minas and Tapira rock phosphates. The difference in behaviour between dicalcium phosphate and the other sources may be explained, in part, by the greater absorption of dicalcium phosphate, leaving a greater part of available P to be used by the animal. In this way, a greater quantity of P is available for the animal and any excess may return to the digestive tract, mainly through saliva, enzymes and digestive juices, as a

Table 4.2. Flow of entrance and exit of P from the central pool to and from the digestive tract, bone and soft tissues in cattle based on the P source (Vitti *et al*., 2001).

	Phosphorus sources		
Variables (g/day)	Dicalcium phosphate	Patos de Minas	Tapira phosphate
P intake	10.9	10.5	10.6
P faeces	6.52[b]	8.28[a]	8.16[a]
P urine	0.001	0.001	0.001
P blood to gatrointestinal tract	10.1[a]	4.81[b]	5.58[b]
P gastrointestinal tract to blood	14.5[b]	7.07[a]	8.05[a]
P blood to tissues (bone and soft)	4.60	4.77	3.35
P tissues (bone and soft) to blood	0.22	2.51	0.88
P availability (coefficient)	0.68[a]	0.46[b]	0.50[b]

Within a row, means with different superscripts differ ($P \leq 0.01$).

regulatory mechanism for this mineral in the blood (Breves and Schröder, 1991). Greater individual variation was observed in the flow of the mineral from the tissues to blood, with even negative values being observed between cattle fed diets with different P sources. This suggests that the reabsorption of P from bone and soft tissues needs to be evaluated better and that it is connected to the total mineral status of the animal and its performance.

It can be seen that the more expensive P source, dicalcium phosphate, had higher P availability, but with values lower than those presented by the NRC (2001), indicating that the amount being offered in the diet is being underestimated. The high levels of fluoride in alternative sources has been another source of worry, principally for the supplementation of dams and sires, which stay longer on the farm, and fluorosis may occur. A study to evaluate superphosphate and Patos rock phosphate for zebu cows over 2 years showed no differences in pregnancy rate, calving rate and calving interval. Fluorine deposition in bone was higher for these P sources, but the superphosphate was almost as good as dicalcium phosphate in provision of phosphorus for grazing cows and there was a potentially significant economic advantage over dicalcium phosphate (Vitti *et al*., 1995).

Phosphorus and endoparasites

Worm infection is one of the most important sanitary aspects in cattle kept at pasture and it may interfere with P utilization. Nevertheless, the great difficulty in measuring damage caused by endoparasites is the wide variety of agents that affect ruminants and the mode of action of the parasites. The parasites may affect the animal directly through the mucosa lining used in absorption or secretion, or indirectly through loss of appetite and consequently DM intake.

More than 70% of parasites recovered from the intestine of tracer calves kept in naturally infected pastures belonged to the nematode *Cooperia*, and more than 92% of these belonged to the species *C. punctata* (Lima, 1998). The fixation site is the upper part of the small intestine, which is also the main site of dietary P absorption (Schröder *et al*., 1995). Thus, damage caused in the intestinal epithelium by parasites could interfere with P absorption and metabolism. Wilson and Field (1983) and Bown *et al*. (1989) observed low P absorption in lambs infected with parasites of the *Tricostrongylus* genus, which occupies the same place in the intestine.

Recently, Rodrigues (2004) carried out two experiments with *Bos taurus* calves infected with *C. punctata* in a single infection and another with a chronic infection to evaluate P metabolism, using the radiophosphorus technique. In the first experiment no loss of calf appetite was observed, and the infection resulted in lower LW gain, level of P in the plasma and P retention. There was also higher P excretion in faeces, caused by mucosal lesions in the small intestine (Rodrigues *et al*., 2004). Although no significant differences were found in terms of endogenous loss and P absorption between healthy and infected calves, these seem to be the mechanisms involved in the explanation for greater P excretion in faeces and lower retention in infected calves. In the second trial, the infected calves

showed a lower appetite, which affected feed intake and P absorption and retention. In this case, there was an overlap of the effects of *C. punctata* beyond the lesions in the intestinal mucosal lining, which is the indirect action reducing feed intake. The chronic infection simulates animals at pasture, and the severity of the process resulted in lower mineral retention (4.31 g P/day) compared with healthy calves. With the acute infection the difference in retention between healthy and infected calves was 1.71 g P/day.

In the field, endoparasitosis is generally associated with more than one species of parasite, and it is clear that they interfere mainly in mineral metabolism, thereby increasing P requirements. This fact should be taken into consideration in herds reared extensively. Phosphorus is one of the most studied minerals in ruminant nutrition worldwide, but important questions about its requirements need to be answered. Phosphorus is a non-renewable element and is responsible for environmental contamination and therefore its use should be optimized.

Phosphorus Utilization in Sheep

Phosphorus sources and availability

A series of experiments with sheep was carried out to determine the endogenous faecal loss and the true P absorption from different P sources (Vitti *et al*., 1991). The sources tested were dicalcium phosphate, bonemeal, monoammonium phosphate, superphosphate, acidulated phosphate, Patos rock phosphate and Tapira rock phosphate. Endogenous P made up 20% of the total P in faeces in sheep fed diets containing rock phosphates and 45% in those fed other sources. The highest true P absorption values were diets containing superphosphate (0.70), followed by bonemeal (0.67), dicalcium phosphate (0.62) and monoammonium phosphate (0.59). Both rock phosphates had true P absorption values of 0.44 and the lowest value was for diets supplemented with acidulated phosphate.

Calculations based on conventional balance technique (P intake – P excretion) for apparent P absorption gave different rankings of P sources. Bonemeal (0.42) and superphosphate (0.39) were ranked highest, followed by the rock phosphates (0.32). Dicalcium phosphate had a value of 0.29 and the lowest-ranking source was acidulated phosphate. Phosphorus absorption values from conventional methods were underestimated because endogenous P losses are not considered. Although P from dicalcium phosphate is considered to be almost 100% available for ruminants in the literature, the isotopic dilution technique shows that actual values are lower (between 0.6 and 0.7; Lofgreen, 1960; Vitti *et al*., 1991). Values reported by Lofgreen (1960) for true P digestibility in sheep were 0.50 and 0.46 for dicalcium phosphate and bonemeal, respectively. These values are lower than the results observed by Vitti *et al*. (1991). Godoy and Chicco (2005), using the radioisotope technique to measure true P absorption in sheep, obtained values of 0.76, 0.71, 0.58 and 0.54 for dicalcium phosphate, superphosphate and two sedimentary phosphates, respectively. Most values of

true P absorption of rock phosphates are lower than for dicalcium phosphate or superphosphate. Arrington *et al*. (1963) showed that P from dicalcium phosphate is absorbed and retained in larger amount than that of rock or soft phosphate. Phosphorus from some rock phosphates may also be highly available, but the utilization in diets is limited due to fluorine content.

The reason for the relative unavailability of some P sources in general has not been identified. Whether the difficulty lies in the problem of solubility and absorption or in the ability of the animal body to utilize what is absorbed is not known (Ammerman *et al*., 1957). Some phosphates are capable of forming polymers of various lengths (Hendricks, 1944) and if this occurs in the intestines, absorption might be impaired. Variations in true availability among similar P sources obtained with the same methodology could be due to the origin and material processing.

Effects of dietary phosphorus levels on phosphorus metabolism

Quantitative aspects of P metabolism in sheep fed different levels of P have been considered using balance studies with the isotope dilution technique (Vitti *et al*., 2000, 2008; Dias *et al*., 2007). Experiments were carried out with 5-month-old (23 kg) Brazilian Santa Inês breed male sheep (exp. 1). The treatments consisted of the basal diet supplemented with different amounts of dicalcium phosphate (0, 8.3, 16.6 and 25 g) to provide 0, 1.5, 3.0 and 4.5 g P/animal/day (Dias *et al*., 2007). Another experiment (exp. 2) used 8-month-old (33 kg) Santa Inês male sheep (Vitti *et al*., 2008). The protocol used was the same as previously described. The treatments consisted of a basal diet supplemented with different amounts of dicalcium phosphate to provide 0, 2, 4, and 6 g P/animal/day.

According to the standard classification scheme (NRC, 2007), the P requirement for sheep of a LW between 20 and 30 kg is 4.0–5.1 g/animal/day. Therefore, P levels were classified as low (< 3.0 g), adequate (3.1–5.7 g) and in excess (> 5.8 g). Plasma P was in the normal range (4–6 mg/dl; NRC, 2007) and additional P had no effect on P in plasma and urine. Although no values were observed below 4 mg P/dl, the use of inorganic P in plasma as an indicator of P status in ruminants is the subject of divergent opinions among researchers, as mentioned earlier. Plasma P is influenced by many factors, such as intake, bone metabolism, starvation and time of collection (Silva Filho *et al*., 2000). Urine P was low, representing less than 2% of P intake.

Phosphorus intake had a linear relationship with faeces P (Eqns 4.2 and 4.3). A similar relationship was also reported by Vitti *et al*. (2000) and Dias *et al*. (2006) working with lambs and goats. Total P excreted in faeces represented an average of about 75% of P intake in both experiments. Values of faecal loss as high 95% of total P intake have been reported (Salviano and Vitti, 1996).

$$P_{faeces} = 0.71\ P_{intake} + 0.88 \text{ (exp. 1, } r^2 = 0.88, n = 12, P < 0.001) \quad (4.2)$$

$$P_{faeces} = 0.86\ P_{intake} - 0.08 \text{ (exp. 2, } r^2 = 0.92, n = 24, P < 0.001) \quad (4.3)$$

Table 4.3. Effect of dietary P concentrations on P metabolism in sheep. Experiment 1 used 5-month-old sheep (23 kg) and experiment 2 used 8-month-old sheep (33 kg).

Experiment 1 (Dias *et al.*, 2007)

	Treatments (g P/animal/day as dicalcium phosphate)			
Parameters (g/day)	0	1.5	3.0	4.5
P intake	0.91[d]	2.55[c]	4.43[b]	6.59[a]
P plasma (mg/dl)	6.80[a]	8.89[a]	9.05[a]	7.29[a]
P in faeces	1.40[d]	2.90[c]	4.10[b]	5.50[a]
P urine	0.003[a]	0.004[a]	0.006[a]	0.005[a]
Endogenous P in faeces	0.70[b]	2.00[a]	2.70[a]	2.90[a]
Dietary P in faeces	0.70[b]	0.80[b]	1.40[b]	2.60[a]
P absorbed from diet	0.20[c]	1.70[b]	3.00[a]	3.90[a]
P availability (coefficient) (%)	0.23[b]	0.67[a]	0.68[a]	0.60[a]
Retained	–0.50[b]	–0.30[b]	0.30[a,b]	1.10[a]

Experiment 2 (Vitti *et al.*, 2008)

	Treatments (g P/animal/day as dicalcium phosphate)			
Parameters (g/day)	0	2.0	4.0	6.0
P intake	1.47[d]	3.53[c]	5.69[b]	7.47[a]
P plasma (mg/dl)	6.66[a]	7.91[a]	8.78[a]	9.02[a]
P in faeces	1.10[d]	3.18[c]	4.40[b]	6.55[a]
P urine	0.0034[a]	0.006[a]	0.025[a]	0.125[a]
Endogenous P in faeces	0.60[c]	1.60[b]	2.41[a]	2.43[a]
Dietary P in faeces	0.50[c]	1.58[b]	1.99[b]	4.12[a]
P absorbed from diet	0.97[c]	1.95[b]	3.52[a]	3.36[a]
P availability (coefficient) (%)	0.66	0.55	0.62	0.45
Retained	0.37[b]	0.44[b]	1.11[a]	1.12[a]

Within rows, different superscripts denote significantly different ($P < 0.05$).

The endogenous faecal loss of P increased with increased P intake. For 5-month-old sheep (Table 4.3), the endogenous loss represented 65% of P intake and the relationship between those variables was:

$$\text{Endogenous P} = 0.31\ P_{intake} + 0.78\ (r^2 = 0.64,\ n = 12,\ P < 0.05) \quad (4.4)$$

This equation indicates that the endogenous loss at zero P intake is 0.78 g/day or about 18 mg P/kg LW/day. This minimum value represents the inevitable loss that would occur if sheep were fed to meet their maintenance requirement. This value is in agreement with the recommendation of NRC (1985) (20 mg P/kg LW/day) and it is higher than the values indicated by ARC (1994) (9–12 mg P/kg LW/day). Assuming the mean coefficient of absorption of 0.65 (Table 4.3), the animal must consume 1.28 g total P/day (0.78/0.65) to meet maintenance requirement.

The endogenous loss in exp. 2 represented 41% of P intake (Table 4.3) and endogenous P was also related to intake:

$$\text{Endogenous P} = 0.31\ P_{intake} + 0.36\ (r^2 = 0.62,\ n = 24,\ P < 0.05) \quad (4.5)$$

The calculated endogenous loss was 0.36 g P/day or 12 mg/kg LW/day for 8-month-old sheep. The coefficient of absorption was 0.57; therefore, the corresponding P intake for maintenance is 0.63 g P/day. This indicated that age affected the minimum endogenous loss and that younger sheep needed a higher P intake for maintenance. More information on endogenous P excretion and true P absorption is required to calculate P requirements accurately, which would mitigate P pollution from animal agriculture.

Phosphorus Utilization in Goats

There is little information in the literature concerning the nutritional requirements of goats, probably because of the minor economic importance of goats in developed countries. Most recommendations have been extrapolated from those of cattle and sheep (e.g. INRA, 1988; AFRC, 1991). However, these extrapolations may be misleading as it is well recognized that goats have developed unusual physiological features in comparison with other ruminants, such as a higher capacity to degrade cell wall components of low-quality forages and a better ability to use nitrogen and water in a marginalized environment (Prieto *et al*., 1990). In addition, the existing published information on P metabolism in goats is relatively sparse and inconsistent (e.g. Akinsoyinu, 1986; Muschen *et al*., 1988). There is very little quantitative information on many indices of P metabolism, such as faecal endogenous P losses and true absorption. Variation in P intake might affect P utilization and homeostasis, and low P intakes might result in low P levels in the blood and soft tissue P pools of the body. An adequate supply of P in the diet for goats depends on the chemical form of P in the diet, level of Ca, level of other minerals and status of the animals. The requirement for total P intake in goats has been estimated by a factorial method, taking into account the physiological needs and a coefficient of absorption. The calculations have been conducted using the latest collection of goat data where possible (Meschy, 2002) and using sheep and cattle data to make adjustments where necessary (NRC, 2007).

P requirements for the maintenance of goats vary between 1.4 and 2.5 g/animal/day (NRC, 2007). Phosphorus balance studies with radioactive P were performed with 14-month-old (15 kg) dwarf goats (Akinsoyinu, 1986). Total minimum endogenous P loss was calculated to be 19.2 mg/kg LW/day. Based on a calculated absorption coefficient of 74%, goats would need 26 mg P/kg LW/day in their diet to meet their maintenance requirements. Bueno (1997) used Alpine goats (34.7 kg) to study P metabolism with the radioisotope technique. The animals were fed hay and a concentrate mixture either without P supplementation or with different amounts of dicalcium phosphate to give 1.8 or 2.6 g P/animal/day for 28 days.

Total faecal P, absorbed P, endogenous faecal P and efficiency of absorption were positively related to P intake, in agreement with other studies (Akinsoyinu,

1986; Bueno and Vitti, 1999). Total P in faeces was higher than P intake for unsupplemented and low P supplemented animals, so they were in negative balance. However, endogenous faecal loss represented about 65% of total P in faeces. Based on calculations of endogenous P, true P absorption was 0.3, 1.07 and 1.95 g P/animal/day for goats fed unsupplemented, low and high P supplemented diets, respectively.

The minimum net endogenous P loss (which represents the loss at zero P intake) was 10.4 mg/kg LW/day, which is nearly half of that reported by Akinsonyinu (1986). It follows that a minimum of 10.4 mg/kg LW must be absorbed daily by goats to meet their maintenance needs. With an absorption coefficient of 66% (mean for the treatments with P supplementation), the animals would need a daily P intake of 15.8 mg/kg LW to meet their maintenance requirements. Queiroz *et al*. (2000) used the factorial method of ARC (1980) and a comparative slaughter technique to determine the daily dietary requirements for maintenance of growing Alpine goats (18 kg), receiving a diet supplemented with dicalcium phosphate (0.79, 1.26 and 1.63 g P/day). The minimum endogenous P loss was calculated to be 8.84 mg/kg LW.

Using data from balance and kinetics experiments with radiophosphorus to trace the movement of P in the body, Carvalho *et al*. (2003) studied the effect of increasing P intake on P utilization in Saanen goats, 4–5 months old and weighing 20–30 kg. The goats received a diet consisting of a concentrate mixture and hay. Phosphorus supplementation was offered as dicalcium phosphate to give 0.42 (low P), 1.36 (medium P) and 3.63 g P/day (high P) (AFRC, 1991). For the low P level, the diet was not supplemented with any additional P. As expected, total P excreted in faeces increased linearly with P intake and represented 53 and 64% of P intake in goats fed medium and high P diets, respectively. Goats fed low P diets were in negative balance. Urinary loss of P was low for all treatments, representing less than 1% of dietary P for the three treatments. Ruminants usually excrete negligible amounts of P in urine, but considerable variation may be observed between animals, with some animals excreting a quite high proportion of P intake in urine (Manston and Vagg, 1970). Urinary loss of P is normally not directly related to P intake, but it is associated with a greater efficiency of absorption and when plasma P concentration exceeds a renal threshold (between 2.0 and 3.0 mM of P) (Challa *et al*., 1989). When P requirements for maintenance and growth are met, P is eliminated in urine (Challa *et al*., 1989). Dietary P levels were not sufficient to provide an excess of P, so P in urine was low for all treatments.

Endogenous faecal P excretion linearly increased with P intake. Previous studies with ruminants have shown that endogenous P in faeces is directly related to intake (Braithwaite, 1985; Challa *et al.*, 1989; Louvandini and Vitti, 1994). Despite a need to retain P, especially in the P-deficient diet, the growing goats were unable to avoid the inevitable endogenous loss through faeces. Total endogenous losses represented 54% of P intake for animals fed unsupplemented diet. For goats fed medium and high P supplemented diet, endogenous P represented about 30% of P intake.

True P absorption was calculated to be 4.58, 34.9 and 96.5 mg P/kg LW/day in low, medium and high P supplemented diets, respectively. Whereas P was

truly absorbed from the low P diet at only 22% efficiency, for medium and high P diets, P was absorbed at 71 and 73% efficiency, respectively, which was similar to the values of 64–70% reported by AFRC (1998). Meschy (2002) also reported that the true P absorption coefficient is higher in goats than in sheep and cattle (70–75%). The improvement in efficiency of absorption obtained when a low P-level diet was supplemented with P was also observed by Braithwaite (1985) for growing lambs fed with deficient and adequate diets. The reason for the low P absorption on a non-supplemented diet may have been due to the low availability of P, mainly present in the organic form. The increased total P absorption was a result of increasing both dietary and endogenous P absorption (Braithwaite, 1984, 1985; Ternouth and Sevilla, 1990). However, some authors report that the net absorption efficiency remains constant, irrespective of the dietary intake of P. According to Braithwaite (1984), the efficiency of P absorption depends on both P intake and P demands. The efficiency should be maximal in animals with high demand and receiving low to moderate P diets, and in such animals the active transport mechanism should be stimulated to the maximum. In the present study, the animals had a high demand for growth, and it was observed that the increase in efficiency was lower from the moderate to high levels than from the deficient to moderate levels. Probably, if greater levels of P were offered to the animals, the efficiency of P absorption would have decreased even further. The minimum endogenous P loss was calculated to be 6.93 mg/kg LW, which is lower than the values obtained by Akinsoyinu (1986) (15.6 mg/kg LW) and Bueno (1997) (10.4 mg/kg LW). Total minimum endogenous loss, which includes urinary and minimum faecal endogenous P excretion, was 7.09 mg/kg LW. With a coefficient of absorption of 22% (low P diet), the goats would need 32.2 mg P/kg LW/day. Based on the mean value of the absorption coefficient for supplemented diets, the animals would need much less to meet their maintenance requirements (9.85 mg P/kg LW/day). According to Pfeffer (1989), the requirement for maintenance in goats can be estimated by the follow equation: P (g/day) = 0.081 + 0.88 DMI, where DMI is dry matter intake in kg/day. Using this equation, P requirement for the goats is 0.72 g P/day, which is similar to the value for a low P diet based on endogenous P determination.

In addition to high P absorption coefficients, goats have better P recycling through saliva (Meschy, 2000) and higher P concentration in saliva (Kessler, 1991). This could explain the low values of P requirements observed by Bueno (1997) and Carvalho *et al.* (2003).

Calcium Utilization

Calcium and P are the two most plentiful minerals in the animal body, being present mainly in bone and teeth as hydroxyapatite crystal. Ninety-nine per cent of Ca and 80% of P are found in bones and teeth, with the remainder in soft tissues and fluids of the body. These minerals help to regulate normal processes of the body and are present in a variety of inorganic and organic compounds distributed within extracellular and intracellular compartments (NRC, 1985;

Whitney and Rolfes, 1996). When dietary intakes are less than required, the minerals are reabsorbed from bone to overcome the deficiency (Braithwaite, 1983).

The remaining 1% of the total Ca in the body is found in soft tissues and body fluids and exists as free ions, bound to plasmatic proteins, especially albumin, or in the form of complexes of organic and inorganic acids. Ionized Ca represents between 50 and 60% of the total Ca and participates in processes of muscular contraction and transmission of nervous impulses and acts as a cofactor for many enzymatic reactions. Ca homeostasis is regulated by hormones to maintain a narrow range of concentration for neuromuscular action. The physiological effects of the hormones on extracellular P are considered to be secondary (Andriguetto *et al*., 1993; Hurwitz, 1996).

The intra- and extracellular Ca is regulated by bidirectional transport in a very narrow system through the cells of the plasmatic membrane and intracellular organelles, e.g. endoplasmic reticulum, sarcoplasmic reticulum in the muscle cell and mitochondria (Merck, 2004). The concentration of intracellular Ca regulates a variety of cellular processes by the activation of protein kinase and phosphorylation enzymes. Calcium is also involved in the action of other intracellular messengers, such as cyclic adenosine monophosphate (cAMP) and inositol 1,3,5-phosphate, and regulates the cell responses to various hormones, including epinephrine, glucagon, vasopressin, secretin and cholecystokinin (Merck, 2004).

The metabolic route of Ca in the animal organism involves intake, digestion, intestinal passage, in which Ca is absorbed via the trans-epithelial membrane, and endogenous faecal excretion. When Ca ion is absorbed, it is quickly mixed with body fluids. If levels of Ca in plasma are normal, about 50% goes to bones, replacing Ca deposited earlier, in a constant exchange between blood and bone tissues (Bronner, 1987). Approximately 50% of plasma Ca that circulates in kidneys is filtered in the tubules, and 70% of that can be reabsorbed in different parts of the kidneys.

It is generally thought that the Ca:P ratio in diet for ruminants should be between 1:1 and 2:1 for the optimum utilization of these two elements (Challa, 1986). However, conflicting results have been reported. For example, Wise *et al*. (1961) and Ricketts *et al*. (1970) reported that ratios greater than 2:1 lead to decreased absorption of P while other workers reported that a Ca:P ratio of less than 1 has similar effects (Vipperman *et al*., 1969). On the other hand, no deleterious effects were observed when Ca:P ratios varied between 1:1 and 10:1 (Young *et al*., 1966; Rajaratne *et al*., 1990). It is suggested that the adverse effects of wide Ca:P ratios on P absorption may only be critical when P intake is inadequate (Young *et al*., 1966). If the diet is rich in Ca, P solubility in the rumen is depressed and dietary P availability is also reduced (AFRC, 1991). According to NRC (1985), the acceptable safe range is a Ca:P ratio of 1:1 to 2:1.

Normally, forages contain an adequate concentration of Ca (Underwood and Suttle, 1999) and it is unusual for grazing ruminants to be subjected to a shortage of Ca, except when heavy supplementary feeding is practised (CSIRO, 2007). In tropical countries, ruminants are commonly fed forage diets that are adequate in Ca but deficient in P, giving an inadequate Ca:P ratio. Thus, it is important to understand the effects of the relationship of Ca level and sources on P and Ca homeostasis control.

Sheep

Salviano and Vitti (1996) studied the effects of Ca:P ratio on P utilization using the isotope dilution technique. Cross-bred male sheep (mean weight 34.4 ± 3.1 kg) were used to study the effect of three Ca:P ratios (0.75:1, 1.5:1 and 3:1). Phosphorus supplementation was offered as sodium phosphate (Na_2HPO_4) to give 3 g P/day. Calcium supplementation ($CaCO_3$) was offered in different amounts to give 2.3, 4.5 and 9.0 g Ca/day, corresponding to Ca:P ratios of 0.75:1, 1.5:1 and 3:1, respectively. Diets consisted of *Cynodon dactylon* hay, cassava meal, urea and trace-mineralized salt, with 8.7% crude protein and 2200 MJ/kg metabolizable energy. Phosphorus supply was kept constant and represented an adequate level. The daily Ca supply was close to the targeted amount and provided deficient (2.3 g/day), adequate (4.3 g/day) and excessive (8.4 g/day) amounts of Ca.

There was no significant effect of Ca level on total P excretion, P excreted in urine and endogenous faecal P excretion. Total P excretion was high, representing about 89% of P intake, which is higher than values reported by other researchers (e.g. Braithwaite, 1985; Louvandini and Vitti, 1994). Endogenous P losses (1.6, 2.0 and 1.78 g/animal/day) were about 60% of P intake and 61% of total P excreted. P absorbed and the coefficient of absorption were 1.7, 2.2 and 1.9 g/animal/day and 0.56, 0.70 and 0.63, with significant difference ($P < 0.05$) between the 1.5:1 and 0.75:1 Ca:P ratios for both parameters. Phosphorus absorption increased by 35% when the Ca:P ratio increased from 0.75:1 to 1.5:1. However, further increase in Ca:P ratio to 3:1 led to a decrease of P absorption by about 25%. Results suggest that wide Ca:P ratios such as 3:1 or ratios less than 1 lead to decreased P absorption, which are in agreement with many studies (e.g. Vipperman *et al.*, 1969; Challa, 1986; AFRC, 1991). Decreased P absorption with excess dietary Ca may be due to hormonal control. The excess dietary Ca may cause above-normal plasma Ca concentrations and thus PTH secretion is inhibited and calcitonin secretion is stimulated. In response to increased calcitonin, bone resorption is blocked and tubular reabsorption of P and Ca is decreased (Care *et al.*, 1980).

In contrast with grazing animals, when ruminants are fed cereal grain diet for extended periods, the addition of Ca supplement is recommended. Cereal grains such as maize have a low Ca level (0.02%) (Andriguetto *et al.*, 1993). Calcium carbonate is the most common form of Ca included in diets for ruminants and it is available as limestone, seashells and coral. Limestone is the cheapest and most common Ca source (NRC, 2007). However, there are other Ca sources used to supplement diets which might affect its metabolism.

Calcium metabolism was studied in Santa Inês Brazilian breed male sheep, aged 8 months and averaging 31.6 kg (Vitti *et al.*, 2006; Dias *et al.*, 2007). The animals were fed diets based on NRC (1985) recommendations for growing lambs, with dry matter intake equal to 4.3% of animal live weight (LW). The diet had a Ca:P ratio of about 2:1 and 13% crude protein content. The basal diet, containing hydrolysed sugarcane bagasse, maize (ground grains), soybean meal, urea, monoammonium phosphate and mineral mixture, was supplemented with different sources of Ca. The treatments were diets with Ca sources from citrus

pulp (CTP), lucerne hay (LUC), limestone (LIM), oyster shell meal (OSM) and dicalcium phosphate (DCP) (Table 4.4). For the study, the animals were injected with radioactive Ca (^{45}Ca) and P (^{32}P) to determine endogenous losses and true availability.

Calcium levels in plasma were in the expected range of 9–12 mg/dl (McDowell, 1992) and were not affected by levels of Ca consumed. Therefore, it cannot be considered a reliable indicator of Ca status (Care *et al*., 1980), except for significant hypocalcaemia cases (particularly for ionized Ca). Plasma Ca concentrations are maintained through the hormonal system by parathyroid hormone (PTH), calcitonin and the active metabolite of vitamin D, 1,25-dihydroxyvitamin D (Care *et al*., 1980; McDowell, 1992). Although there were differences in Ca intake in the treatments, they did not impair metabolism and Ca was maintained above 0.51% of DM intake for all treatments.

Total Ca in faeces was lower for LIM treatment and represented about 59% of Ca intake. For other Ca sources, faecal Ca was about 90% of intake. The presence of pectin in CTP could have affected its absorption. Giraldo (1999) reported that citrus pulp has 19% pectin on a DM basis. According to Fernandez *et al*. (2002), pectin can be bound to cation such as Ca and could impair its absorption. The low Ca availability in LUC could be due to the presence of oxalate (Fredeen, 1989). In lucerne hay, 20–33% of total Ca is present as insoluble oxalate and apparently unavailable for the animal (Ward *et al*., 1979).

Endogenous Ca loss in faeces was about 18, 12, 20, 12 and 11% of Ca intake in CTP, LUC, LIM, OSM and DCP, respectively. These were within the range of 16–20% Ca endogenous loss reported by Martz *et al*. (1990). Endogenous

Table 4.4. Mean values of variables related to calcium metabolism in lambs fed different calcium sources (Vitti *et al*., 2006; Dias *et al*., 2007).

	Calcium sources[a]					
Variables	CTP	LUC	LIM	OSM	DCP	SE[b]
Live weight (kg)	31.9^{a}	32.9^{a}	31.3^{a}	30.8^{a}	31.6^{a}	4.5
Ca in plasma (mg/dl)	12.2^{a}	10.1^{b}	10.7^{a,b}	9.98^{b}	9.33^{b}	0.80
DM intake (g/kg LW/day)	39.1^{a}	35.5^{a,b}	29.7^{b,c}	36.4^{a}	28.8^{c}	2.93
Ca intake (mg/kg LW/day)	209^{a,b}	197^{a,b}	169^{b}	219^{a}	194^{a,b}	18.6
Faecal Ca (mg/kg LW/day)	205^{a}	196^{a}	98.0^{c}	179.0^{a,b}	142^{b,c}	20.6
Faecal endogenous Ca (mg/kg LW/day)	39.0^{a}	23.1^{b}	34.2^{a,b}	26.2^{a,b}	22.0^{b}	6.68
Ca net absorption (mg/kg LW/day)	42.8^{c,d}	24.2^{d}	105^{a}	66.1^{b,c}	74.2^{b}	11.6
Ca biological availability (%)	20.5^{c,d}	12.5^{d}	62.1^{a}	30.3^{b,c}	39.7^{b}	5.43
Ca in urine (mg/kg LW/day)	3.13^{a}	1.00^{a}	1.75^{a}	2.26^{a}	4.09^{a}	2.21
Ca retention (mg/kg LW/day)	0.65^{c}	0.003^{c}	69.9^{a}	37.7^{b}	48.0^{a,b}	10.8

[a]CTP = citrus pulp; LUC = lucerne hay; LIM = limestone; OSM = oyster shell meal; DCP = dicalcium phosphate.

[b]SE, standard error of mean.

Means followed by different superscripts, within rows, differ by Duncan test ($P < 0.05$).

Ca in faeces can be influenced by the efficiency of reabsorption of secreted Ca from the gut; however, this is not confirmed by experimental evidence (AFRC, 1991). Endogenous Ca faecal loss can be related to Ca intake. For example, Dorigan (2000) found increased values of endogenous Ca faecal loss (10.1, 12.7 and 18.2 mg/kg LW/day) for sheep receiving increasing amounts of Ca in diet (0.06, 0.17 and 0.30% Ca, respectively). However, according to the ARC (1980), endogenous Ca loss in ruminants is constant (16 mg/kg LW/day). According to Braithwaite (1981, 1982), there is a linear relationship between Ca endogenous loss and DM intake. The same author found values of up to 43.2 mg Ca/kg LW/day in sheep consuming 60 g DM/kg LW/day. Biological availability from diet LIM was 62.1%, which was close to the 68% considered by AFRC (1991) and Dorigan (2000) as the coefficient for Ca absorption. The values of bioavailability reported for LUC (20.5%), LIM (62.1%) and DCP (39.7%) were different from those observed by Hansard *et al*. (1957). The authors used diets supplemented with Ca from 15 organic and inorganic sources. The mean availability was higher for young animals than for adults, and for the first category the mean values for lucerne hay, limestone and dicalcium phosphate were 41.0, 45.0 and 58.5%, respectively.

Urinary Ca excretion was low (about 2%), irrespective of the amount of Ca ingested, and it could be considered an obligatory loss (Cunningham, 1993). Braithwaite and Riazuddin (1971) and Braithwaite (1979) observed low amounts of Ca excreted in urine of sheep, with values up to 8 mg/kg LW/day. Ca retained by animals receiving the LIM diet was higher than for those with the other treatments. Ca retention for CTP and LUC approached zero because of higher endogenous losses in relation to absorption. Limestone showed the highest Ca true digestibility among treatments and is therefore considered adequate for supplementing diets for growing lambs. It is suggested that LUC and CTP should not be used as an exclusive Ca source in lamb diets due to their low true Ca digestibility.

Phosphorus metabolism was studied with CTP, LIM, LUC and OSM treatments. All treatments supplied adequate amounts of P according to NRC (1985). Phosphorus intake was 3.2, 4.2, 4.2 and 4.4 g/day for LIM, LUC, CTP and OSM, respectively. Urinary loss of P presented significant differences between treatments. Animals fed LIM excreted 0.32 g/day, whereas animals fed LUC and CTP excreted 0.02 and 0.03 g/day, respectively. Urinary losses of P are generally low in ruminants, but considerable variation is found among animals (Manston and Vagg, 1970; Field *et al*., 1984). The physical form of the diet was reported to affect the pathway of P excretion (Scott *et al*., 1984b).

Total endogenous losses represented 50.6, 45.5, 44.2 and 36.9% of total faecal P excreted for LIM, LUC, CTP and OSM treatments, respectively. Endogenous faecal P losses were, on average, 44% of total P excreted in faeces, which is lower than the 66% reported previously in cattle and sheep (Coates and Ternouth, 1992; Bortolussi *et al*., 1996). The difference could be related to the low P availability in the different supplements tested. Scott and Buchan (1985) compared the effects of feeding either roughage or concentrate diets on salivary secretion and urinary P excretion in sheep and observed that animals that consumed roughage secreted more saliva and excreted less P in urine. Differences in P excretion through urine may also be due to the saturation capacity of salivary

glands to clear the plasma P, which was high. Truly absorbed P was: 41.4, 36.5, 32.6 and 40.1% for LIM, LUC, CTP and OSM, respectively ($P > 0.05$). True phosphorus absorption was considered low for all treatments and this could be due to the form of P present in the diet, mainly as phytate. Phosphorus retention was negative for treatments LIM, LUC and CTP (–0.48, –0.95 and –1.0 g/day) and positive for OSM (0.06 g/day). Although all treatments supplied adequate amounts of P, the low absorption led to negative retention. The high P demand of the growing animals could also have caused the negative values for P retention.

Goats

The effect of Ca intake on Ca metabolism was studied in Saanen goat kids (Dorigan, 2000). The diet was based on whole crushed maize and straw (54%), crushed maize grains (39%), soybean meal (5.5%) and minerals. The animals received rations containing three levels of Ca as limestone (0.06, 0.17 and 0.30% of dry matter) and similar levels of crude protein (9%), energy and P (0.25% of dry matter). The animals were intravenously injected with radio calcium (^{45}Ca) for determination of endogenous Ca and true absorption. Average Ca intake was 22.8, 53.4 and 93.3 mg/kg LW/day for the three levels studied, and this affected faecal Ca excretion, faecal endogenous Ca losses, plasma inorganic Ca, Ca absorption and Ca retention (Table 4.5). Calcium in faeces represented 85, 61 and 50% of Ca intake for dietary Ca levels of 0.06, 0.17 and 0.30%, respectively. Biological availability of Ca was not affected by the treatments (mean = 65.2%). The biological availability of Ca for the basal ration was 63% and for the limestone it was 74%. Endogenous faecal Ca was affected by the treatments and varied between 10.1 and 18.2 mg/kg LW/day. Endogenous Ca values were 44.0, 23.8 and 19.5% of intake for the three Ca levels studied. The minimum Ca requirement for maintenance was calculated to be 9.71 mg/kg LW/day.

Table 4.5. Mean values and standard errors (in parentheses) of variables related to calcium metabolism in Saanen goats fed different calcium levels (Dorigan, 2000).

	Ca level in diet (% DM)		
	0.06	0.17	0.30
Ca intake (mg/kg LW/day)	22.8 (1.05)^{a}	53.4 (2.57)^{b}	93.30 (3.58)^{c}
Ca plasma (mg/dl)	9.83 (1.25)^{b}	11.5 (1.38)^{ab}	13.58 (2.50)^{a}
Ca urine (mg/kg LW/day)	1.18 (0.38)^{b}	1.62 (0.52)^{b}	2.41 (1.26)^{a}
Ca faeces (mg/kg LW/day)	19.31 (4.22)^{c}	32.4 (2.41)^{b}	46.69 (6.17)^{a}
Ca faeces (%)	0.48 (0.05)^{a}	0.85 (0.09)^{b}	1.12 (0.20)^{b}
Endogenous Ca (mg/kg LW/day)	10.05 (2.75)^{b}	12.7 (1.95)^{b}	18.16 (4.01)^{a}
Ca absorbed (mg/kg LW/day)	14.51 (2.40)^{a}	33.7 (3.02)^{b}	64.76 (5.55)^{c}
True absorption (%)	63.00 (8.13)^{a}	63.15 (5.42)^{a}	69.34 (4.37)^{a}
Ca retention (mg/kg LW/day)	3.07 (2.29)^{c}	18.40 (1.86)^{b}	44.20 (6.61)^{a}

Means followed by different superscripts, within rows, differ by Tukey's test ($P < 0.05$).

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5 Phosphorus and Calcium Utilization in Non-ruminants Using Isotope Dilution Technique

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Introduction

An adequate supply of P and Ca in monogastrics is essential due to their structural, physiological, catalytic and regulatory functions in the body, and to maintain adequate mineral reserves (Underwood and Suttle, 2001). Determination of P and Ca requirements to ensure animal health and productivity accurately, while minimizing nutrient excretion in manure, requires understanding maintenance requirements, endogenous losses and utilization of dietary P and Ca intakes. The isotope dilution technique has been used to study P in swine, equines and poultry, and is described in detail in this chapter. However, Ca utilization studies using the isotope dilution technique in monogastrics are scarce. In this chapter, Ca utilization in horses is discussed.

Phosphorus Utilization in Swine

Adequate supplementation of phosphorus (P) in pig diet contributes to a faster and more efficient rate of growth, better development of bones and lower feed cost, reduces P excretion and mitigates environmental pollution (Cavalcante, 1994). Determination of P requirement in pigs requires an understanding of P availability from different sources and at different levels of consumption. The evaluation of biological P availability for P requirement calculations involves the determination of faecal and urinary endogenous P excretion (Akinsoyinn, 1986;

Fernandez, 1995). Although there are several techniques for determination of biological P availability, the radioisotope technique can be more accurate in measuring true absorption and distribution of P in tissues (Vitti, 1989).

Lopes *et al*. (1999a) conducted a study using growing pigs fed diets containing different levels of total P (0.30, 0.40, 0.51, 0.65 and 0.73%), using the isotope dilution technique. The diet containing 0.30% P in this study was unsupplemented and the other diets were supplemented with various amounts of dicalcium phosphate. Linear relationships were established between P intake and P excreted in faeces, urine and endogenous P with slopes of 0.23, 0.15 and 0.023, respectively. The faecal endogenous P excretion at zero P intake (inevitable endogenous loss) was 5.85 mg/kg LW/day, which is in the range of 2.9–24.5 mg/kg LW/day reported by Jongbloed (1987) for pigs weighing 15–140 kg (with P in the diet ranging from 0.33 to 0.83%). Phosphorus intake was also linearly related to P retention and absorption, with a slope of 0.62. Based on the mean P absorption coefficient for diets supplemented with dicalcium phosphate, endogenous faecal P and urinary P loss at zero P intake (18.1 mg/kg LW/day), the minimum total P intake for maintenance was calculated to be 38.6 mg/kg LW/day. In another experiment with finishing pigs, Lopes *et al*. (1999b) observed that P excreted in faeces and urine was linearly related to P intake, with a slope of 0.24 and 0.04, respectively. The authors calculated the faecal endogenous P loss to be 4.27 mg/kg LW/day, which was close to the 3.2 mg/kg BW/day reported by Petersen and Stein (2006), who used a semi-purified diet.

A wide range of values has been reported in the literature for endogenous P loss in pigs. Schulin-Zeuthen *et al*. (2007) conducted a meta-analysis and found that a diminishing return type of equation was best suited to describe the relationship between P retention and intake and therefore to calculate endogenous P excretion. The authors calculated it to be 14 mg/kg $BW^{0.75}$/day, based on reported available P values, and 17 mg/kg of $BW^{0.75}$/day, based on total P values, which was very close to that reported by Rodehutscord *et al*. (1998), who showed endogenous P excretion to be 15.5 mg of P/kg of $BW^{0.75}$/day, based on regression analysis of 66 P balance studies. Variable endogenous P loss values were reported for growing pigs fed a semi-purified diet (e.g. 7.3–9.3 mg/kg of $LW^{0.75}$/day; Pettey *et al*., 2006), a maize-based diet (0.67 g/kg DM intake or 30.2 mg/kg of LW/day; Shen *et al*., 2002) and a soybean meal-based diet (0.45 g/kg of DM intake or 20.3 mg/kg of LW/day; Ajakaiye *et al*., 2003). Dilger and Adeola (2006) summarized estimates of endogenous P excretion values reported in the literature. They concluded that the endogenous P loss in pigs was likely to be less than 20 mg/kg of $BW^{0.75}$/day.

Sources of dietary phosphorus for swine diets

Generally, dicalcium phosphate is used as source of inorganic P supplementation. Despite large deposits of rock phosphate in countries such as Brazil, it has not been used as a source of P. However, there are restrictions to the use of these rocks due to the high fluorine content (Rezende *et al*., 1999) and due to lack of information regarding P absorption.

Figueirêdo *et al*. (2001) studied P metabolism in pigs fed diets based on maize and soybean meal and supplemented with different sources of P (dicalcium phosphate, Tapira rock phosphate, Patos rock phosphate, monoammonium and triple superphosphate). A control group receiving only the basal diet without P supplementation was set up for comparison. P intake was not influenced by the phosphate sources (mean 7 g/day) but pigs fed the control diet consumed less feed (3 g/day). The total P excretion in faeces did not differ between the diets supplemented with different sources of inorganic P (mean = 47.2% of P intake). Pigs fed the control diet excreted a lower amount of P but higher in proportion to intake (59.1%). True P absorption and retention were also not different between P sources, in agreement with Bellaver *et al*. (1984), who did not observe differences on P absorption from different sources (Goias rock phosphate, Patos rock phosphate, Tapira rock phosphate). There was no difference between the biological availability of P of dicalcium phosphate and that of rock phosphates. Teixeira *et al*. (2004) also studied P metabolism in pigs fed diets with different P sources. Apart from urine P excretion, the authors did not find a significant difference in absorbed and retained P between dicalcium phosphate and other sources of P. Therefore, they argued that different sources of P may be a good alternative to dicalcium phosphate when reserves diminish and the cost of inorganic P rises. An example of a P distribution model based on Fernandez (1995) and adapted by Lopes *et al*. (2001) is shown in Fig. 5.1.

Phosphorus Utilization in Equines

Proper growth of the skeleton is vital to the healthy development of young horses. Impediments to normal development of the skeleton can seriously impair the horse's ability to perform to its maximum genetic potential. Nutritional factors have been linked with 'developmental orthopedic disease' in young horses (Teeter *et al*., 1967; Cunha, 1991). Although several nutritional factors have been associated with causing the disease, P deficiency has been shown to be critical for the development and integrity of the skeleton in growing horses (Hintz and Schryver, 1972; Hintz *et al*., 1976). In spite of its importance in skeletal development, other factors need to be considered when trying to achieve the correct levels of P in food sources. Studies have concluded that exercise of the horse also influences the mineralization of bones and bone quality. Moreover, P oversupplementation can cause undesirable imbalances among minerals (Honoré and Uhlinger, 1991) and can be expensive (Furtado, 1996).

Common horse feedstuffs that are relatively high in P include cereal grains, oilseed meals (cottonseed meal and soybean meal) and some alternative feeds such as sugarcane yeast. However, most of the organic phosphate in feeds is in the form of phytate, which is relatively unavailable for absorption in non-ruminants. Several studies have investigated the digestion and metabolism of P in horses (e.g. Schryver *et al*., 1971; Kichura *et al*., 1983), in which P bioavailability ranged from 30 to 45%. Hintz (1983) reported 32% and 40% P bioavailability in maize grain and oat grain, respectively. Phosphorus bioavailability can also be

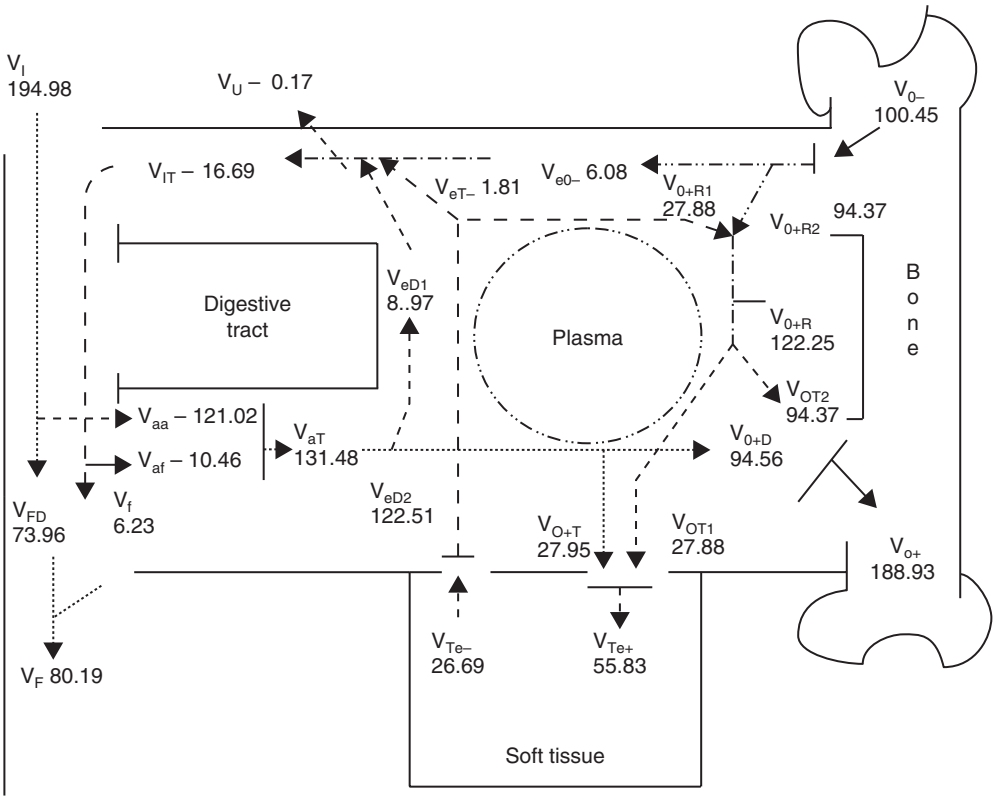

Fig. 5.1. Phosphorus (P) distribution model for growing pigs fed a diet containing Tapira phosphate and a daily intake of 194 mg P/kg LW/day. The abbreviations are as follows: V_I, P intake; V_F, total faecal P excretion; V_f, endogenous faecal P; V_{FD}, faecal P of dietary origin; V_u, urinary P excretion; V_{aa}, absorbed P of dietary origin; V_{IT}, endogenous P origin input to gastrointestinal tract (GIT); V_{af}, endogenous P reabsorbed into the GIT; V_{aT}, total absorbed P; V_{0+}, P accretion in the bone; V_{0-}, reabsorbed P in the bone; V_{O+R2}, P from bone recycled to bone and soft tissues; V_{e0-}, reabsorbed P from the bone into the GIT; V_{O+D}, P from V_{aT} accretion to the bone; V_{Te+}, P accretion in the soft tissues; V_{Te-}, reabsorbed P from the soft tissues; V_{0+R1}, P from the soft tissues recycled into bone and soft tissues; V_{O+R}, total P recycled into bone and soft tissues; V_{eT-}, reabsorbed P from the soft tissues into the GIT; V_{O+T}, P from V_{aT} to accretion in the soft tissues; V_{eD1}, P from V_{aT} into the GIT; V_{eD2}, P from V_{aT} into bone and soft tissues; V_{0T1}, P from V_{0+R} accretion into the soft tissues; V_{0T2}, P from V_{0+R} accretion into bone (Lopes *et al*., 2001).

influenced by P intake, age of the animals and Ca:P ratio (Schryver *et al*., 1971); however, studies that quantify the effect are scarce.

According to Georgievskii (1982), P bioavailability can be determined by P adioisotope studies. Furtado *et al*. (2000a) evaluated the effect of different P sources on P metabolism in growing horses by determining their biological P availabilities. The authors evaluated Tapira rock phosphate, Patos rock phosphate,

dicalcium phosphate and bonemeal added to a basal diet to supply 22 g P/animal/day. The animals were injected with 30 MBq ^{32}P/animal and specific activities of plasma, faeces and urine were determined and faecal endogenous loss and true P absorption were calculated. They reported that P intake, excretion, plasma concentration and retention were unaffected by phosphate sources. Reported P availability values were 25.2, 33.9, 31.7 and 29.4% for Tapira rock phosphate, Patos rock phosphate, dicalcium phosphate and bonemeal, respectively, which were not significantly different from the commonly used source (dicalcium phosphate).

In a separate experiment, Furtado *et al*. (2000b) studied the effects of different P levels in the diet of growing horses on the endogenous faecal loss and P availability. They used three treatment levels (no P supplementation and dicalcium phosphate supplementation to give 15, 20 and 25 g P/day). Faecal endogenous loss and true P absorption were calculated, based on specific activities of the radiophosphorus in the plasma, faeces and urine, for each horse in the experiment. A linear relationship between P intake and P excretion was established. About 73% of dietary P intake was excreted in faeces, confirming that the main route of P excretion in horses was through faeces. There was also a linear relationship between absorbed P, retained P and dietary P intake. Urine P and faecal endogenous loss were reported to be unaffected by P levels.

Quadros (2006) studied the effect of Ca on P metabolism, using the isotope dilution technique, in growing equines. They used three levels of dietary Ca intake (0.15, 0.45 and 0.75% of diet), with same level of P (0.23% of diet), and did not find significant effects on P intake, plasma P, P in faeces, endogenous P in faeces, absorbed P and retained P. They concluded that P level in the diets met the requirement of the horses in the experiment and P metabolism was not affected within a Ca:P range of 0.65:1 to 3.2:1.

Another study on P metabolism was carried out using the isotope dilution technique to determine the P bioavailability of feeds in Brazil, using 12-month-old horses (Oliveira *et al*., 2008). Five diets were formulated to contain approximately equivalent levels of crude protein and digestible energy and to supply the NRC (1989) recommended level of at least 22 g P/horse/day. All five diets contained 40% Bermuda coastal hay plus 60% concentrate. The concentrates were maize + cottonseed meal (C1), maize grain + soybean meal (C2), maize + sugarcane yeast (C3), oat + cottonseed meal (C4) and oat + soybean meal (C5). Although all P diets resulted in a positive P retention, P intake was lowest in horses fed diet C3 (79.7 mg/kg LW). Absolute values of P concentrations in plasma, urine and faeces were similar in all treatments as well as endogenous P loss. Phosphorus bioavailability values were 50.8, 41.0, 43.5, 51.0 and 57.7% for diets from C1 to C5, respectively, and were significantly different between diets C2 and C3 and the other diets. Bioavailability values of all dietary treatments exceeded NRC (1989) recommendations of 35% true P absorption in diets not supplemented with inorganic P. The results of this study indicate that inorganic P supplementation may not be required for growing yearlings fed common Brazilian feeds, and, taking into account cost and risk of environmental P contamination, the issue needs serious consideration and further confirmation.

Phosphorus Utilization in Poultry

Data on P utilization using the isotope dilution technique in poultry are very scarce. Conte (2000) evaluated P availability in broiler chickens fed diets containing whole rice bran supplemented with three levels of phytase (400, 800 and 1200 FTU/kg). The birds were given peritoneal ^{32}P, and faecal endogenous excretion, apparent and true absorption and plasma P were measured. The authors reported that all variables increased with increasing levels of phytase. They determined that the bioavailability of P in rice bran was 28.1%, which increased to 38.3, 50.2 and 54.0% with phytase supplementation of 400, 800 and 1200 FTU/kg, respectively. Furthermore, Conte (2000) reported that phytase-supplemented birds had higher ash and P in tibia, reduced concentrations of P, Zn, Mn and Cu in the excreta and higher uptake of P, Zn, Mn and Cu.

Calcium Utilization in Equines

Studies of calcium (Ca) metabolism in horses using the isotope dilution technique are scarce. Furtado *et al*. (2009) studied Ca metabolism in growing equines using the isotope dilution technique by feeding 12 male horses aged 1 year (221 kg LW) diets with different levels of Ca and injecting ^{45}Ca into the jugular vein of the animals. The authors did not observe any differences in plasma, urine and endogenous faecal Ca between treatments and the average values were 11.8, 5.54 and 20.9 mg Ca/kg LW/day, respectively. The average biological availability of Ca was 81.7% and no effects were found among the horses fed various levels of Ca. A strong linear relationship between Ca intake and absorbed Ca, total Ca in faeces and retained Ca was established (Furtado *et al*., 2009).

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6 Bioavailability of Calcium and Phosphorus in Feedstuffs for Farm Animals

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Abstract

Dietary calcium (Ca) and phosphorus (P) are never fully utilized by animals as some may be in an unavailable form, lost during normal digestive and metabolic processes, or their absorption negatively influenced by several dietary and/or non-dietary factors. Thus, meeting the requirements for these elements for the various classes of farm animals depends on their bioavailability in the feedstuffs used. Furthermore, formulating diets on bioavailable calcium Ca and P basis minimizes their output in the manure, which has economic and ecological consequences. The bioavailability of Ca and P from feedstuffs is determined either as digestible P or as available P, using digestibility or the slope ratio assays, respectively. Except for legume forages, vegetable feedstuffs are very low in Ca content and therefore provision of adequate dietary Ca supply is almost entirely achieved through the use of animal-based and inorganic feedstuffs whose Ca bioavailability is > 66%. Vegetable feedstuffs have very variable P content (cereals and their by-products, 2–27, oilseed meals, 6–12 g/kg dry matter), the majority (> 65%) of which is phytate-bound and unavailable to the animal without enzymatic dephosphorylation. Phytase, the requisite enzyme to hydrolyse phytate, is insufficient in avian and mammalian intestinal secretions, present in some feedstuffs and ubiquitous in microbial systems. As a consequence, P bioavailability in vegetable feedstuffs is higher for ruminant than for non-ruminant species. Research and commercial efforts to increase utilizable P in vegetable feedstuffs for non-ruminants include dietary inclusion of microbial phytase, use of low phytate and higher intrinsic phytase grains and oilseed meals, and the development of phytase-secreting transgenic animals. Phosphorus in most of the animal-based and inorganic feedstuffs is highly available (> 50%). Determination of Ca and P bioavailability in feedstuffs is influenced by many factors, including experimental, dietary and animal, and as result a given feedstuff may be assigned different bioavailability

values. This may present challenges in deciding the most appropriate value applicable for diet formulation.

Introduction

Calcium and P are the most abundant mineral elements in the body and are often the major cations in the diet. In the body, 99% of Ca and 80% of P reserves are located in the skeleton (bones and teeth). The remaining 1% of Ca and 20% of P reserves are extremely important for the biochemical processes and as constituents of the organic components. The requirement for these elements varies markedly throughout an animal's lifespan (NRC, 1994, 1998, 2001). Quantitatively, their role in the development and maintenance of the skeletal system is their most important function and their accumulation in the skeletal tissue is interdependent, as one element will not accumulate without the other (NRC, 2005). Meeting the requirement of Ca and P for an animal depends on their bioavailability in the feedstuffs (Coffey, 1994).

Except for diets high in legume forages for ruminants, vegetable feedstuffs are very low in Ca content (NRC, 2005). Instructively, very little is known about the Ca availability in vegetable feedstuffs, perhaps a consequence of the fact that most of these feedstuffs contribute so little Ca to the diet such that its bioavailability is of little consequence (NRC, 2005). For example, on a dry matter basis, maize contains 0.3 g/kg and soybean meal (SBM) 3.2 g/kg; hence, the Ca contributed by these two feedstuffs in a typical maize-SBM diet for finishing pigs is only about 0.6 g/kg against a requirement of 4.5 g/kg (NRC, 1998). Furthermore, 20–30% of Ca in plant tissues is bound to oxalate, which is relatively unavailable (NRC, 2001). Consequently, provision of an adequate Ca supply in the diet is almost entirely achieved through animal-based and inorganic feedstuffs; of which the common sources include ground limestone, mono- and dibasic calcium phosphate, calcium chloride and oyster shells (NRC, 2005).

Phosphorus nutrition has been studied more extensively than most other mineral elements because it is a much more costly mineral (Soares, 1995). Vegetable feedstuffs, inorganic supplements and bone-, meat, poultry and fish-meals are the major sources of P for animals (NRC, 2005). On a dry matter basis (g/kg), the P contents of vegetable feedstuffs are: forages (2.3–3.5), oilseed meals (5.7–12), cereals (2.3–4.1) and cereal by-products (8.0–27) (NRC, 2005). Over 65% of the P in these feedstuffs is phytate-bound and unavailable to the animal without enzymatic dephosphorylation.

Phytase, the requisite enzyme to hydrolyse phytate, is present in some feedstuffs; it is insufficient in avian and mammalian intestinal secretions and ubiquitous in microbial systems (Selle and Ravindran, 2007). Arguably, P bioavailability in vegetable feedstuffs is higher for ruminant than for non-ruminant species due to phytate dephosphorylation by rumen microbial phytase (NRC, 2001). Consequently, to provide adequate P for non-ruminant species, it is necessary to include feedstuffs with high P availability such as inorganic supplements (e.g. dicalcium phosphate) or animal-based feedstuffs (e.g. meat and bonemeal) in the diet. While this approach is plausible for adequate P nourishment,

it creates three challenges: excessive excretion of P in the manure; expensive diets; and considerable demand for non-renewable global reserves of rock phosphate (Selle and Ravindran, 2007). In cognizance of the fact that the extent of use of supplemental sources of P in diets for non-ruminants depends on the bioavailable P content of the basal vegetable feedstuffs, research and commercial efforts have been directed at improving utilization of phytate in these feedstuffs. To this end, strategies such as dietary inclusion of microbial phytase, vegetable feedstuffs with low phytate content, maize expressing extra phytase and transgenic pigs expressing salivary phytase have been evaluated (Golovan *et al*., 2001; Raboy, 2002; Nyannor *et al*., 2007).

The present chapter will address the methodologies used to evaluate bioavailability of Ca and P, available data on their bioavailability in feedstuffs and factors influencing bioavailability estimates.

Methods for Estimating Bioavailability of Calcium and Phosphorus

Bioavailability of Ca and P is determined either as digestible or as available, using slope ratio or digestibility assays, respectively (Nyachoti and Johnston, 2006).

Slope ratio

The assay provides a combined estimation of digestion and post-absorptive utilization at the tissue level (Ammerman, 1995). A nutrient bioavailability is determined based on the effectiveness of a given source, relative to that of selected, highly available sources, in improving a set response criterion. The procedure involves feeding graded levels of Ca or P from a test feedstuff below the requirement to induce an experimental deficiency response. These values are then assessed relative to response from a known standard that is presumed to be 100% available. The response criterion is plotted against Ca or P intake of animals fed the test feedstuff diets and for those fed the standard diets, and regression equations are calculated (Cromwell, 1992). The slope ratio is then calculated by dividing the slope of the regression equation from the test feedstuff by the slope of the regression equation of the standard feedstuff to obtain the estimate of relative bioavailability for the test feedstuff.

This technique has been used to determine Ca and P bioavailability in a variety of poultry and swine feedstuffs but rarely used in ruminant studies (Ammerman, 1995). Values obtained from this method are considered the 'gold standard' and are widely used in formulation of feeds for non-ruminant species (NRC, 1994, 1998). In the slope ratio method, the choice of a sensitive response criterion that can be easily measured is especially important (Coffey, 1994).

Growth performance in young animals has been used as the primary criterion for determining bioavailability of mineral elements; however, it becomes less satisfactory in mature and large animals because of dietary and labour costs (Ammerman, 1995). Bone development has been considered for a long time as

one of the most critical tests for estimating Ca and P bioavailability because large amounts of these elements are used for this purpose (Ammerman, 1995). Utilization of bone measurements follows the principle that bone can be tested as either a structure or a material (Johnston, 2008). Bone breaking strength and breaking load relate to bone structure while bone ash and bone scan measurements relate to material characteristics of total bone mineral content (Crenshaw *et al*., 1981; Johnston, 2008). Biochemical indices including 1,25-dihydroxyvitamin D_3, parathyroid hormone, alkaline phosphatase, osteocalcin and hydroxyproline, and blood mineral levels are also routinely used to assess the mineral nutrition status of animals (van Mosel and Corrlet, 1990; Alexander *et al*., 2008).

Digestibility methods

The slope ratio method has various drawbacks that limit routine application (De Lange, 2000; Nyachoti and Johnston, 2006). Because of ease of determination, measures of digestible Ca and P content in feedstuffs are routinely used in formulating diets as reasonable estimates of bioavailability (Nyachoti and Johnston, 2006). The term digestibility is used interchangeably with absorption in the literature. The mineral must be absorbed from the gastrointestinal tract (GIT), and the assumption is made that, once absorbed, the element is available for storage or for use in various physiological processes by the animal (Ammerman, 1995). Thus, digestibility, unlike the slope-ratio assay, does not consider post-absorptive utilization of Ca and P at the tissue level (Fan *et al*., 2001). Collection of ileal digesta and/or faeces is necessary for determination of the digestibility coefficient (Adeola, 2001). Based on whether the measurement takes into account the contribution of endogenous Ca and P losses, the digestibility coefficient can be designated as either apparent or true.

Apparent

Current digestible Ca and P values used in practical feed formulation are referred to as apparent digestible values because they are not corrected for Ca and P from endogenous sources. Apparent digestibility coefficients are variable among studies for the same feed ingredient and, because of the confounding effect of endogenous losses, apparent Ca and P digestibility values underestimate the amount of Ca and P that is truly digested (Nyachoti and Johnston, 2006). Thus, the ability to formulate diets accurately with respect to Ca and P supply is limited when using apparent digestibility values.

True

Determination of true Ca and P digestibility entails experimental quantification of endogenous losses using appropriate radioisotopes and stable isotopes (Hansard *et al*., 1957; Martz *et al*., 1999), Ca- and P-free diets (Ajakaiye *et al*., 2003a; Petersen and Stein, 2006), regression analysis (Fan *et al*., 2001) and substitution (Ammerman *et al*., 1957). A measurement of true Ca and P digestibility is a characteristic of a feed ingredient and thus is not influenced by assay conditions. Unlike apparent digestibility, true digestibility valves are additive in a mixture of

feed ingredients, as has been demonstrated for amino acids (Nyachoti *et al*., 1997). None the less, it is important to note that additivity of true Ca and P digestibility in a mixture of feed ingredients has been determined in only a few studies (e.g. Fang *et al*., 2007). Thus far, the true digestible P content has been determined in only a few feed ingredients (Nyachoti and Johnston, 2006).

In vitro methods

In vitro techniques have been promoted as alternatives to animal assays, which are impractical for routine evaluation and screening of feedstuffs or diets because they are time-consuming and expensive (Zyla *et al*., 1995). For instance, Liu *et al*. (1998) developed a technique using maize–soybean meal-based diets, which has been shown to give P availabilities in genetically modified and normal maize that are similar to those obtained with the slope ratio assay (Spencer *et al*., 2000a). In ruminants, numerous studies have been conducted using microbiological techniques, based on the concept that P-depleted rumen bacteria will rapidly digest cellulose only when supplied with adequate amounts of available P. Despite the potential benefits that *in vitro* methods offer in terms of rapid determination of Ca and P availability, their widespread use is yet to be realized (Nyachoti and Johnston, 2006).

Bioavailability of Calcium and Phosphorus in Feedstuffs

Tables 6.1, 6.2, 6.3 and 6.4 give the bioavailability of Ca and P in various feedstuffs as reported in the literature. True digestibility values are also presented and discussed where applicable.

Calcium

The limited data on the availability of Ca in vegetable feedstuffs suggest their inadequacies as Ca sources, especially for non-ruminants. Inorganic and animal-based sources of Ca have high Ca bioavailability values of 80% or more for poultry and swine (Table 6.1). Limestone (calcitic), a common dietary Ca source, has bioavailability values of 92 and 99% for poultry and swine, respectively. The relatively lower bioavailability of the Ca in dolomitic limestone is due to magnesium content, which results in a denser, less soluble crystal (Ross *et al*., 1984). Oyster shell, a common source of Ca in laying bird diets, also has highly (100%) available Ca. Marble dust and aragonite are considered less common sources for Ca for domestic animals (Peeler, 1972). Considerably more research on Ca bioavailability has been reported for poultry than for other animals, underscoring the importance of Ca to these species. For instance, a laying bird consuming 100 g of feed per day requires 3 g of Ca (NRC, 1994).

With respect to ruminants, true digestible Ca for inorganic feedstuffs is 50% or more and generally higher than that for organic sources (Table 6.1).

Table 6.1. Bioavailability of calcium in various feedstuffs.

Species/source	Mean[a]	Min.	Max.	Method	References
Poultry					
Bivalve shell	27	–	–	True digestibility	Ajakaiye *et al.* (2003a)
Bonemeal	100	–	–	Slope ratio	Blair *et al.* (1965)
Calcium carbonate	17	–	–	True digestibility	Ajakaiye *et al.* (2003a)
Calcium gluconate	100	–	–	Slope ratio	Waldroup *et al.* (1964)
Calcium sulfate	95	90	100	Slope ratio	Waldroup *et al.* (1964); Hurwitz and Rand (1965)
Eggshell	100	–	–	Slope ratio	Meyer *et al.* (1973)
Limestone	92	87	100	Slope ratio	Waldroup *et al.* (1964); Reid and Weber (1976)
Limestone, dolomitic	66	–	–	Slope ratio	Stillmark and Sunde (1971)
Marble dust	19	–	–	True digestibility	Ajakaiye *et al.* (2003a)
Oyster shell	19	–	–	True digestibility	Ajakaiye *et al.* (2003a)
Oyster shell	100	100	100	Slope ratio	Waldroup *et al.* (1964); Reid and Weber (1976); Roland (1986)
Rock phosphate, defluorinated	92	90	94	Slope ratio	Dilworth and Day (1964); Burnell *et al.* (1990)
Rock phosphate, soft	68	–	–	Slope ratio	Dilworth and Day (1964)
Swine					
Aragonite	98	–	–	Slope ratio	Ross *et al.* (1984)
Gypsum	98	–	–	Slope ratio	Ross *et al.* (1984)
Limestone	99	–	–	Slope ratio	Ross *et al.* (1984)
Limestone, dolomitic	78	–	–	Slope ratio	Ross *et al.* (1984)
Marble dust	98	–	–	Slope ratio	Ross *et al.* (1984)
Oyster shell, ground	98	–	–	Slope ratio	Ross *et al.* (1984)
Ruminants					
Lucerne hay	58	30	80	True digestibility	Hansard *et al.* (1957); Fredeen (1989); Martz *et al.* (1990)
Maize silage	52	39	52	True digestibility	Martz *et al.* (1990)
Grass hays	44	36	51	True digestibility	Hansard *et al.* (1957)
Milk, dried skim	56	23	89	True digestibility	Lengemann *et al.* (1957)
Aragonite	49	–	–	True digestibility	Wholt *et al.* (1986)
Calcite flow	49	–	–	True digestibility	Wholt *et al.* (1986)
Calcium carbonate	59	40	85	True digestibility	Hansard *et al.* (1957); Goetsch and Owens (1985)
Calcium chloride	63	53	71	True digestibility	Hansard *et al.* (1957); Goetsch and Owens (1985)
Dicalcium phosphate	73	50	73	True digestibility	Hansard *et al.* (1957)
Limestone	50	37	67	True digestibility	Hansard *et al.* (1957)
Monocalcium phosphate	55	49	61	True digestibility	Hansard *et al.* (1957); Tillman and Brethour (1958a)
Rock phosphate, defluorinated	48	40	55	True digestibility	Hansard *et al.* (1957)

[a]Simple average of reported values.

Table 6.2. Bioavailability of P in various feedstuffs for poultry.

Feedstuff	Mean	Min.	Max.	Method	References
Cereal grains and by-products					
Barley	38	32	50	Slope ratio	Harrold *et al.* (1979a); Hayes *et al.* (1979); Coffey (1994)
Barley, brewers' dried grain	32	–	–	Slope ratio	Coffey (1994)
Maize, dry	21	12	33	Slope ratio	Harrold *et al.* (1979b); Huang and Allee (1981); Coffey (1994)
Maize, DDGS	76	69	102	Slope ratio	Lumpkins and Batal (2005); Martinez Amezcua *et al.* (2004); Martinez Amezcua and Parsons (2007); Kim *et al.* (2008)
Maize, gluten feed	95	–	–	Slope ratio	Coffey (1994)
Maize, hominy feed	34	–	–	Slope ratio	Coffey (1994)
Sorghum, dry	26	18	36	Slope ratio	Trotter and Allee (1979a); Huang and Allee (1981); Coffey (1994)
Sorghum, high moisture	47	–	–	Slope ratio	Trotter and Allee (1979a); Coffey (1994)
Oats	42	28	47	Slope ratio	Harrold *et al.* (1979b); Huang and Allee (1981); Hahn *et al.* (1990); Coffey (1994)
Oats, groats	4	–	–	Slope ratio	Coffey (1994)
Oats, bran	60	–	–	Slope ratio	Hahn *et al.* (1990)
Rice	0	–	–	Slope ratio	Coffey (1994)
Rice bran	10	2	18	Slope ratio	Corley *et al.* (1980); Coffey (1994)
Triticale	31	–	–	Slope ratio	Coffey (1994)
Wheat	39	28	58	Slope ratio	Hayes *et al.* (1979); Trotter and Allee (1979b); Huang and Allee (1981); Coffey (1994)
Wheat, middlings	41	–	–	Slope ratio	Coffey (1994)
Wheat, bran	30	23	36	Slope ratio	Corley *et al.* (1980); Coffey (1994)
Oilseed meals					
Canola meal	45	–	–	Slope ratio	Coffey (1994)
Cottonseed meal	42	–	–	Slope ratio	Huang and Allee (1981)
Soybean meal, hulled	16	11	40	Slope ratio	Harrold *et al.* (1979a); Trotter and Allee (1979b); Huang and Allee (1981); Coffey (1994); Sands *et al.* (2003)
Soybean meal, low phytate	57	–	–	Slope ratio	Sands *et al.* (2003)
Soybean meal, hulled	60	–	–	True digestibility	Dilger and Adeola (2006a)
Soybean meal, low phytate	77	–	–	True digestibility	Dilger and Adeola (2006a)
Soybean meal, dehulled	24	16	33	Slope ratio	Coffey (1994)

(*Continued*)

Table 6.2. *Continued.*

Feedstuff	Mean	Min.	Max.	Method	References
Soyabean meal, hulls	119	–	–	Slope ratio	Coffey (1994)
Sunflower meal	23	–	–	Slope ratio	Harrold *et al.* (1983)
Pulses					
Field peas	28	–	–	Slope ratio	Coffey (1994)
Pinto beans	40	–	–	Slope ratio	Coffey (1994)
Animal-based feedstuffs					
Bonemeal	90	89	94	Slope ratio	Gillis *et al.* (1954)
Meat and bonemeal	76	52	99	Slope ratio	Harrold *et al.* (1979a); Huang and Allee (1981); Coffey (1994)
Fishmeal	103	–	–	Slope ratio	Coffey (1994)
Casein	48	–	–	Slope ratio	Harrold *et al.* (1983)
Inorganic					
Curaçao Island phosphate	81	55	100	Slope ratio	Gillis *et al.* (1954); Damron *et al.* (1974)
Dicalcium phosphate	91	71	123	Slope ratio	Peeler (1972); Coffey (1994)
Monophosphates, Na and Ca	96	93	101	Slope ratio	Coffey (1994)
Rock phosphate, raw	75	67	91	Slope ratio	Gillis *et al.* (1954); Godoy and Chicco (2001)
Rock phosphate, defluorinated	89	82	103	Slope ratio	Peeler (1972); Potter (1988); Burnell *et al.* (1990); Coffey (1994)
Rock phosphate, soft	47	25	76	Slope ratio	Gillis *et al.* (1954); Hurwitz (1964)

Table 6.3. Bioavailability of P in various feedstuffs for pigs.

Feedstuff	Mean	Min.	Max.	Method	References
Cereal grains and by-products					
Barley	30	28	31	Slope ratio	Coffey (1994)
Barley, brewers' dried grain	25	16	32	Slope ratio	Coffey (1994)
Maize, dry	18	9	29	Slope ratio	Huang and Allee (1981); Spencer *et al.* (2000b); Coffey (1994)
Maize, low phytate	62	62	62	Slope ratio	Spencer *et al.* (2000b)
Maize, high moisture	46	43	49	Slope ratio	Boyd *et al.* (1983)
Maize, DDGS	77	71	84	Slope ratio	Burnell *et al.* (1989); Coffey (1994)
Maize, gluten feed	59	58	59	Slope ratio	Coffey (1994)
Maize, hominy feed	14	–	–	Slope ratio	Cromwell (1992)
Rice bran	25	–	–	Slope ratio	Cromwell (1992)
Sorghum, dry	26	20	28	Slope ratio	Huang and Allee (1981); Coffey (1994)
Sorghum, high moisture	43	–	–	Slope ratio	Cromwell (1992)

(*Continued*)

Table 6.3. *Continued.*

Feedstuff	Mean	Min.	Max.	Method	References
Triticale	46	–	–	Slope ratio	Cromwell (1992)
Oats	29	22	36	Slope ratio	Stober *et al.* (1980); Huang and Allee (1981); Coffey (1994)
Oats, groats	16	16	16	Slope ratio	Coffey (1994)
Wheat	50	51	49	Slope ratio	Huang and Allee (1981); Coffey (1994)
Wheat, middlings	33	31	34	Slope ratio	Stober *et al.* (1980); Coffey (1994)
Wheat, bran	32	21	41	Slope ratio	Stober *et al.* (1980); Coffey (1994)
Oilseed meals					
Canola meal	15	9	21	Slope ratio	Coffey (1994)
Canola meal	34	34	34	True digestibility	Akinmusire and Adeola (2009)
Canola meal, added phytase	61	–	–	True digestibility	Akinmusire and Adeola (2009)
Cottonseed meal	22	1	42	Slope ratio	Huang and Allee (1981); Cromwell (1992)
Soybean meal, hulled	33	14	73	Slope ratio	Huang and Allee (1981); Ross *et al*. (1982); Ketaren *et al.* (1993a); Coffey (1994)
Soybean meal, hulled	47	41	51	True digestibility	Ajakaiye *et al.* (2003b); Dilger and Adeola (2006b); Akinmusire and Adeola (2009)
Soybean meal, dehulled	23	17	35	Slope ratio	Ross *et al.* (1982); Coffey (1994)
Soybean meal, added phytase	69	66	77	Slope ratio	Ketaren *et al.* (1993b)
Soybean meal, low phytate	63	–	–	True digestibility	Dilger and Adeola (2006b)
Soybean meal, transgenic pigs	93	–	–	True digestibility	Golovan *et al.* (2001)
Soybean meal, hulls	78	–	–	Slope ratio	Ross *et al.* (1982)
Sunflower meal	3	–	–	Slope ratio	Burnell *et al.* (1988)
Pulses					
Field peas	28	6	54	Slope ratio	Ketaren *et al.* (1993a); Johnston (2008)
Field peas	56	51	61	True digestibility	Stein *et al.* (2006); Johnston (2008)
Animal-based feedstuffs					
Meat and bonemeal	73	61	93	Slope ratio	Huang and Allee (1981); Coffey (1994); Traylor *et al.* (2005)
Fishmeal	93	83	102	Slope ratio	Coffey (1994)
Milk products	94	76	115	Slope ratio	Burnell *et al.* (1989); Coffey (1994)
Inorganic					
Curaçao Island phosphate	81	69	93	Slope ratio	Plumlee *et al.* (1958); Coffey (1994)

(*Continued*)

Table 6.3. *Continued.*

Feedstuff	Mean	Min.	Max.	Method	References
Dicalcium phosphate	97	89	107	Slope ratio	Plumlee *et al.* (1958); Peeler (1972); Huang and Allee (1981)
Monophosphates, Na and Ca	100	–	–	Slope ratio	Plumlee *et al.* (1958); Coffey (1994)
Rock phosphate, defluorinated	90	87	92	Slope ratio	Coffey (1994)
Rock phosphate, soft	53	34	72	Slope ratio	Plumlee *et al.* (1958); Coffey (1994)

Table 6.4. Bioavailability of P in various feedstuffs for ruminants.

Feedstuff	Mean	Min.	Max.	Method	References
Lucerne, hay	84	67	94	True digestibility	Lofgreen and Kleiber (1954); Martz *et al.* (1990)
Maize silage	81	75	89	True digestibility	Dayrell and Ivan (1989); Martz *et al.* (1990, 1999)
Wheat bran	26	–	–	True digestibility	Ellis and Tillman (1961)
Curaçao Island phosphate	90	–	–	True digestibility	Ammerman *et al.* (1957)
Defluorinated phosphate	100	–	–	Slope ratio	Miller *et al.* (1987)
Defluorinated phosphate	54	–	–	True digestibility	Ammerman *et al.* (1957)
Dicalcium phosphate	73	50	100	True digestibility	Ammerman *et al.* (1957); Tillman and Brethour (1958b); Lofgreen (1960); Dayrell and Ivan (1989)
Metaphosphates, Na and Ca vitreous	61	34	87	True digestibility	Ammerman *et al.* (1957); Tillman and Brethour (1958a)
Monophosphates, Na and Ca	76	63	89	True digestibility	Ammerman *et al.* (1957); Tillman and Brethour (1958a)
Phosphoric acid	90	–	–	True digestibility	Tillman and Brethour (1958b)
Pyrophosphates, Na and Ca	90	90	90	True digestibility	Ammerman *et al.* (1957); Tillman and Brethour (1958a)
Rock phosphate, raw	48	–	–	True digestibility	Dayrell and Ivan (1989)
Rock phosphate, soft	32	14	50	True digestibility	Ammerman *et al.* (1957); Lofgreen (1960)
Rock phosphate, soft	17	17	17	Slope ratio	Long *et al.* (1956)
Urea ammonium polyphosphate	100	100	100	Slope ratio	Teh *et al.* (1982)

A wide range in true digestibility of Ca in limestone (37–67%) and reagent Ca carbonate (40–85%) in ruminants is associated with its low solubility (NRC, 2005). Lucerne is a major contributor of Ca in ruminant rations, and efficiency of absorption of Ca in lucerne is used to model Ca utilization from forages (NRC, 2001).

Phosphorus

In maize and sorghum, P in dry grains is 17–26% available to swine and poultry and almost two times more bioavailable in high-moisture grains for swine (Tables 6.2 and 6.3). Contrary to the P in dry maize and grain sorghum, wheat and barley have much higher P bioavailability (> 30%) for non-ruminants due to high intrinsic phytase activity (Selle and Ravindran, 2007). Slightly lower P availability in wheat by-products relative to grain wheat could be due to the fact that fibre is known to reduce digestibility (Coffey, 1994). By-products of dry milling (e.g. hominy feed) are relatively low in P bioavailability compared with those originating from wet milling (e.g. maize gluten feed) or fermentation (e.g. distillers' grains). Possible explanations for the discrepancies include activation of phytase by the moisture and/or the microorganisms used in the fermentation and/or steeping processes (Coffey, 1994). However, it is noteworthy that brewers' dried grain has lower bioavailable P compared with dry barley despite the fact that brewing is a fermentation process, perhaps a consequence of different microorganisms not being able to produce extracellular phytase. Surprisingly, oat groats, a product of dehulled oats subjected to a steam rolling process (heat and moisture), show much less P bioavailability compared with intact oats. Rice is the main cereal crop in Eastern and southern Asia and irrigated areas in the tropics and, although very little rice is available for animal usage, a considerable amount of rice milling by-products is available for animal feeds (Kiarie *et al*., 2006). The P availability in rice grain and rice bran is relatively low, which is in line with its high (81%) phytate content (Corley *et al*., 1980).

Soybean meal receives the most usage as a protein supplement in diets for swine, poultry and high-producing dairy cattle (NRC, 1994, 1998, 2001). Bioavailability of P in hulled and dehulled SBM is ~30% for swine and ~20% for poultry (Tables 6.2 and 6.3). Interestingly, P in soybean hulls is highly available (> 75%) for swine and poultry. Since P bioavailabilities in hulled and dehulled SBM are comparable, it may be that the hull fraction of soybeans has a lower level of phytate P compared with other fractions or higher phytase activity as compared with other parts of the bean (Coffey, 1994). Canola meal, a major protein source in canola seed growing regions like western Canada, has a higher bioavailable P content for poultry compared with SBM (Table 6.2).

Genetically modified low-phytate feedstuffs such as maize (Spencer *et al*., 2000a) and SBM (Sands *et al*., 2003) have higher P bioavailability and true digestibility values than their conventional counterparts (Tables 6.2 and 6.3). Similarly, P bioavailability of feedstuffs such as SBM fed with microbial phytase is higher (Ketaren *et al*., 1993b). Almost all P in SBM is truly digestible (93%) in

transgenic pigs expressing more phytase (Golovan *et al*., 2001). Phosphorus from animal-based and inorganic sources is highly (> 70%) available to pigs and poultry (Tables 6.2 and 6.3). However, P in metaphosphates and pyrophosphates is poorly available to non-ruminants, for reasons not well known (Soares, 1995).

True digestibility coefficients for P from lucerne hay or maize silage are > 80% for ruminants (Table 6.4). Such high P digestibility is due to inherent phytase activity of ruminal microorganisms, which renders nearly all of the phytate P available for absorption (NRC, 2001). For dicalcium phosphate, phosphoric acid and monophosphates (Na and Ca), the reference standards in the NRC (2001) model for dairy cattle P nutrition show high (> 70%) true P digestibility. Unlike non-ruminants, P in meta- and pyrophosphates is considerably more available to ruminants (Table 6.4).

Factors that Influence Estimation of Bioavailability

Determination of Ca and P bioavailability is influenced by many factors, some of which are briefly discussed.

Experimental factors

Response criteria

The estimates of the availability of P in most feedstuffs have been shown to be dependent on the response criterion chosen. For instance, Ketaren *et al*. (1993a, b) reported P bioavailability in SBM to be 17 and 61%, respectively, when using bone traits and empty body retention as response criterion. Johnston (2008) determined P bioavailability in peas for swine to range from 6% for plasma P to 29% for bone ash. In this scenario it is challenging to decide the right value to use in diet formulation. However, since the role of these two elements in the development and maintenance of the skeletal system is their most important function, bone trait measurements have been recommended as the most appropriate response criterion (Coffey, 1994; NRC, 1998; Johnston, 2008). Traylor *et al*. (2005) suggested taking an average value when different bone traits are used to determine P bioavailability in a particular feedstuff.

Sample processing

Bone properties, mineral content, density and strength are common indicators for assessing bone quality and they can be influenced by the physical state of the bone (wet or dry) (Orban *et al*., 1993). Procedures require defleshing of bones after excision, drying, extracting fat, weighing and ashing. Although these procedures have been used with a certain degree of accuracy, considerable variation occurs in reported values because of lack of standardized test procedures (Orban *et al*., 1993). A common procedure before processing bones is to dry them, but it is known that differences exists in the physical and mechanical properties of wet and dry bones (Miller *et al*., 1965; Crenshaw *et al*., 1981). Thus, when dry bones are used to assess bone strength or bone mineral content, it is questionable

whether the values obtained reflect the true status of the bones (Orban *et al*., 1993). However, the possibility of conducting bone scans on live animals presents a tremendous opportunity to overcome some of these challenges since they can be performed on a live animal (Crenshaw *et al*., 1981; Johnston, 2008).

Dietary factors

Bioavailability of P in vegetable feedstuffs is largely dependent on phytate content and intrinsic phytase activity within the grain, and these factors may differ greatly between feedstuffs. For instance, Selle and Ravindran (2007) and Steiner *et al*. (2007) summarized data in which phytase activity ranged from 25 to 9945 U/kg in maize and wheat bran samples, respectively. Irrespective of the forms in which Ca and P are ingested, their absorption is dependent on their solubility at the point of contact with the absorbing membranes (NRC, 1998). Absorption of both Ca and P is thus favoured by factors that hold them in solution. The solubility of Ca compounds is favoured by acidic and hindered by alkaline conditions (McDowell, 1992). Calcium is absorbed in the ionic form, and factors that reduce the concentration of ionic Ca (oxalate, phytate, phosphate and excessive sulfate) reduce its uptake in animals (Soares, 1995).

Macro-minerals such as Ca, P, magnesium and potassium interact through competition for uptake at the enterocyte (McDowell, 1992). Therefore, their ratios in the diets may influence their GIT absorption and consequently availability (NRC, 1998). For instance, a wider than optimum Ca:P ratio has significant negative effects on P utilization, especially in diets marginal in P (NRC, 1998). Furthermore, a high dietary Ca concentration is thought to reduce hydrolysis of phytate by endogenous and exogenous phytase as a result of the formation of insoluble Ca–phytate complexes (Selle and Ravindran, 2007).

The form and quantity of vitamin D available to an animal can significantly influence Ca and P absorption in animals (NRC, 2005). This would in turn influence the availability of Ca and P in a feedstuff. Other dietary factors capable of influencing P availability include dietary fibre, which increases endogenous P losses (Coffey, 1994).

Animal factors

Animals absorb Ca from the GIT according to need, and they can alter the efficiency of absorption to meet a change in requirement (Soares, 1995). For instance, it has been shown that young animals absorb more Ca than mature animals (Hansard *et al*., 1957; Horst *et al*., 1978; Braithwaite and Riazuddin, 1971), thus emphasizing the importance of using animals of similar age when comparing the relative availability of Ca in feedstuffs. Endogenous P losses in pigs have been estimated to be 110, 156 and 226 mg/day for 27, 59 and 98 kg pigs, respectively (Pettey *et al*., 2006). Thus, endogenous P loss increases with increasing body weight of the pig by 1.63 mg for every 1 kg increase in body weight, which underscores the need for using animals of the same body weight to estimate true digestible P.

Summary

Total Ca or P in feedstuff does not accurately reflect the proportion that is utilized by the animal. Estimates of available Ca and P content should therefore be determined and used in feed formulation to improve the accuracy of dietary Ca and P supply relative to requirements. A review of available data on estimates of bioavailable Ca and P in common feedstuffs for farm animals has been presented. In general, P in cereal grains and protein supplements derived from plants is poorly available to non-ruminant species. However, microbial phytase, low-phytate feedstuffs and pigs expressing more endogenous phytase, as well as some of the grain processing methods, such as wet milling and fermentation, enhance P availability in vegetable feedstuffs. In contrast to the vegetable feedstuffs, the Ca and P in most feedstuffs of animal origin and inorganic supplements are highly available. Unlike the ruminant species, essentially all the P in metaphosphates and pyrophosphates is unavailable to non-ruminant species.

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7 Phosphorus and Calcium Nutrition and Metabolism

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Abstract

About 98% of the body's calcium (Ca) and 80% of the phosphorus (P) are present in the skeleton as hydroxyapatite. The remaining Ca is in the extracellular fluid and plasma and within the cell, where it has essential roles in metabolism, blood clotting, enzyme activation and neuromuscular function. The remaining P is present in cells as nucleic acids, nucleotides, phospholipids and numerous phosphorylated compounds that function in metabolism. Non-ruminants fed plant-based diets require Ca and P supplementation. Ruminants fed forage-based diets usually do not require Ca or P supplementation, while ruminants fed high-concentrate diets may require supplementation. The phytate in grains and oilseed meals reduces the digestibility of P (and to a lesser extent Ca) for non-ruminants, making P availability in the non-ruminant diet an important consideration. Conversely, P availability is not important in ruminant diets because the rumen bacteria hydrolyse phytate. A narrow Ca:P ratio increases the efficiency of P absorption in non-ruminants. However, the Ca:P ratio is not important for ruminants when the diet is adequate in Ca and P. The apparent absorption of Ca and P occurs primarily in the small intestine of non-ruminants and ruminants by passive and active transport, with small amounts absorbed in the rumen, omasum and abomasum of ruminants and the caecum of swine. In horses, the colon is the major site of P absorption. For ruminants, the recycling of endogenous P from saliva into the rumen may provide over half of the P required by the microorganisms. Plasma Ca^{2+} and P concentrations are controlled within a narrow physiological range by feedback mechanisms involving parathyroid hormone, activated vitamin D_3 (calcitriol), calcitonin and their respective receptors in the small intestine, bone and kidney. When plasma Ca^{2+} and/or P are too low, parathyroid hormone followed by calcitriol are released to increase plasma Ca^{2+} and/or P by intestinal absorption and bone resorption and to reduce Ca^{2+} and/or P excretion by the kidney. Conversely, when plasma Ca^{2+} and/or P are too high, the peptide

hormone calcitonin reduces the intestinal absorption and the bone resorption of Ca^{2+} and P and increases excretion by the kidney. In conclusion, Ca and P homeostasis is maintained by feedback mechanisms regulated by plasma Ca^{2+} and P concentrations, which trigger the release of hormones that affect intestinal absorption, bone apposition or resorption and kidney excretion of Ca^{2+} and P.

Introduction

Calcium (Ca) is the major cation required in the mammalian diet, and is the most abundant mineral element in the body. The skeleton, an articulated framework that facilitates locomotion and provides some support for the vital internal organs, contains about 98% of the body Ca as calcium phosphate. The remaining Ca, about 2%, is distributed in the extracellular and cellular fluids, and has essential roles in metabolism, blood clotting, enzyme activation and neuromuscular function (Soares, 1995a; Pond *et al*., 2005). The metabolism of Ca and phosphorus (P) is closely related, and a deficiency or an excess of either one will interfere with the utilization and metabolism of the other (Kebreab and Vitti, 2005).

Phosphorus is second only to Ca in abundance in the body, with about 80% of the body P located in the skeleton, the remaining 20% having essential metabolic functions in cell contents and cell walls. Phosphorus functions as a component of the nucleic acids which are the basis of genetics, and in nucleotides, such as adenosine triphosphate (ATP), which function in energy metabolism (Soares, 1995b). Phosphorus is a component of both cell walls and cell contents as phospholipids and phosphoproteins. In addition, P functions in acid–base buffer systems of blood and body fluids, in cell differentiation and in maintaining the structural integrity of cells (NRC, 2001; Kebreab and Vitti, 2005).

Non-ruminants fed grain–oilseed meal-type diets require Ca and P supplementation because these feed ingredients are deficient in Ca, and the phytate in cereal grains and oilseed meals reduces the availability of Ca and P for poultry (Farkvam *et al*., 1989; Li *et al*., 2001) and swine (Näsi, 1990; Veum *et al*., 2007; Veum and Ellersieck, 2008). For ruminants, however, most of the phytate is hydrolysed by the ruminal bacteria (Yanke *et al*., 1998; Kincaid and Rodehutscord, 2005). Growing and adult ruminants fed forage-based diets generally do not require Ca or P supplementation. Feedlot cattle and lactating dairy cows fed diets high in concentrates may or may not require Ca or P supplementation, based on the level of production, forage quality and forage type (grass versus legume) because the Ca content of grass is only half that of legume forages (NRC, 1996, 2001).

The primary source of Ca for diet supplementation is ground limestone (also known chemically as $CaCO_3$), because more than 80% of the Ca in the earth's crust exists as limestone. Phosphorus does not occur free in nature because it is extremely unstable and reactive. Therefore, all P compounds found in nature are phosphates, and as orthophosphates in igneous rock (McDowell, 2003). Phosphorus supplements are expensive compared with Ca supplements, making the content and availability of P in feedstuffs an important consideration in diet formulation, especially for non-ruminants. Therefore, proper Ca and P nutrition for farm animals is dependent upon adequate Ca and P in the diet, the availability

of P and the Ca:P ratio for non-ruminants, sufficient sun-activated or dietary vitamin D, and healthy organs for the production of the essential hormones that function in Ca and P metabolism and the conversion of vitamin D to the metabolically active form required by the non-ruminant species (Hoenderop *et al*., 2005; Schröder and Breves, 2007; DeLuca, 2008).

Absorption of Calcium and Phosphorus

Factors that affect the absorption of Ca and P from the digestive tract include the dietary concentrations of Ca and P and the Ca:total P ratio, which should normally be within the range of 1:1 to 2:1 for broilers, turkeys and swine (NRC, 1994, 1998). Egg-laying poultry are an exception, where the Ca:P ratio may reach 4:1 or greater to achieve adequate eggshell development (Fig. 7.1). A narrow Ca:total P ratio of approximately 1:1 increases the efficiency of P absorption and increases the bone strength of broiler chicks (Sebastian *et al*., 1996; Qian *et al*., 1997) and growing swine (Nielsen *et al*., 1971; Liu *et al*., 1998) compared with wider Ca:total P ratios. High dietary Ca from supplemental calcium carbonate increased colonic pH, which reduced the degradation of phytate in the colon of swine (Sandberg *et al*., 1993). For ruminants, however, when the diet is adequate in Ca and P, the Ca:P ratio is not important in the utilization of Ca and P for milk production or in maintaining Ca and P homeostasis (Stevens *et al*., 1971; NRC, 1996, 2001). An exception is that a Ca:P ratio of 2:1 or greater is recommended to prevent urinary calculi in sheep (NRC, 1985).

The apparent absorption of Ca and P occurs primarily in the duodenum and jejunum in the small intestine of non-ruminants (Partridge, 1978; Liu *et al*., 2000; R&D Systems Inc., 2008) and ruminants (Scott *et al*., 1984; NRC, 2001; Pfeffer *et al*., 2005), although *in vitro* studies also found significant absorption of

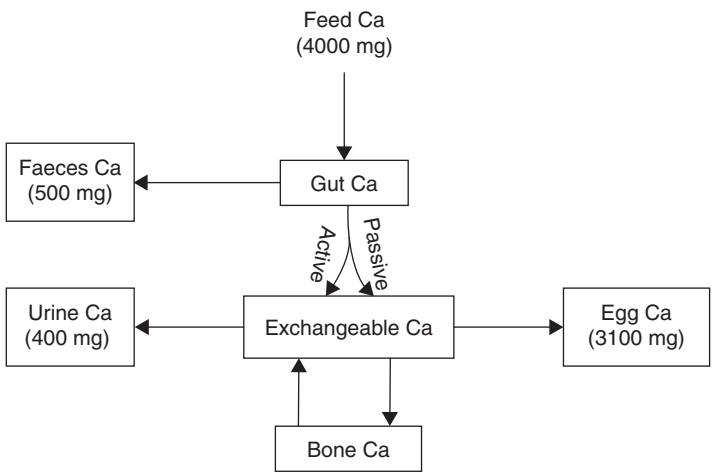

Fig. 7.1. Schematic representation of Ca flow in the laying hen (based on Leeson and Summers, 2001).

Ca^{2+} in the rumen of small ruminants (Schröder *et al*., 1997). For swine, small amounts of Ca and P are absorbed in the caecum with little to no absorption in the colon (Liu *et al*., 2000), whereas the colon is the primary site of P absorption in horses (Frape, 2004). For ruminants, only small amounts of P are absorbed from the rumen, omasum and abomasum (Yano *et al*., 1991; NRC, 2001). The percentages of Ca and P that were absorbed declined with increasing intake (Fernández, 1995a). Calcium absorption occurs both by diffusion (passive transport) and by active transport (energy-dependent transport), with about half the Ca absorbed in the small intestine by each process (Hurwitz, 1996; Pond *et al*., 2005). The regulation of Ca and P absorption also occurs in the small intestine, with less regulation of P than of Ca. For non-ruminants, vitamin D must be converted to the biologically active form $(1,25(OH)_2D_3$, also named calcitriol) to enhance Ca and P absorption and metabolism (NRC, 1994, 1998; DeLuca, 2008). In ruminants, the absorption of P occurs independently of vitamin D, and excessive Ca has no effect on the efficiency of P absorption. This uncoupling of Ca and P absorption in ruminants allows for the maximum absorption of P during a period of P deficiency, and simultaneously reduces the absorption of Ca when Ca is not needed (Pfeffer *et al*., 2005).

In adult, non-lactating ruminants, the P needs of the rumen microbes may exceed the requirements of the host animal (Preston and Pfander, 1964). The recycling of endogenous P from saliva (mostly ionic P) into the rumen provides at least half of the P that the microbes need, as the concentration of P in ruminal saliva is four to 16 times greater than that in plasma (Scott, 1988; NRC, 2001; Kincaid and Rodehutscord, 2005). Dietary P is the only other source of P entering the rumen. Rumen bacteria generally produce adequate amounts of the phytase enzyme needed to hydrolyse phytate, so ruminants with a healthy, functional rumen do not have the P availability problems experienced by non-ruminants (Clark *et al*., 1986; Yanke *et al*., 1998; Kincaid and Rodehutscord, 2005). Figure 7.2 summarizes P flow through the digestive tract and its interaction with blood and bone P in the ruminant.

A kinetic model developed for whole-body P metabolism in growing goats fed different concentrations of dietary P, with ^{32}P injected intravenously to follow the movement of P in the body, found that P absorption, bone resorption, urinary and faecal P excretion and endogenous P excretion were all involved in the homeostatic control of P. Also, goats fed the P-deficient diet had significant endogenous P losses, resulting in a negative P balance (Vitti *et al*., 2000). For swine, bone resorption or accretion was independent of Ca and P intake, whereas endogenous faecal Ca and P increased with increasing P intake (Fernándaz, 1995b), with similar bone and endogenous P responses observed in goats (Vitti *et al*., 2000). Diets containing high concentrations of Ca and P may impair normal bone development in swine by inhibiting bone resorption (Fernández, 1995c).

Physiological Control of Calcium and Phosphorus

Extracellular calcium and phosphorus concentration and function

Extracellular ionic Ca^{2+} has essential 'first messenger' functions after binding to specific receptors on target cells. These functions include the transmission of nervous

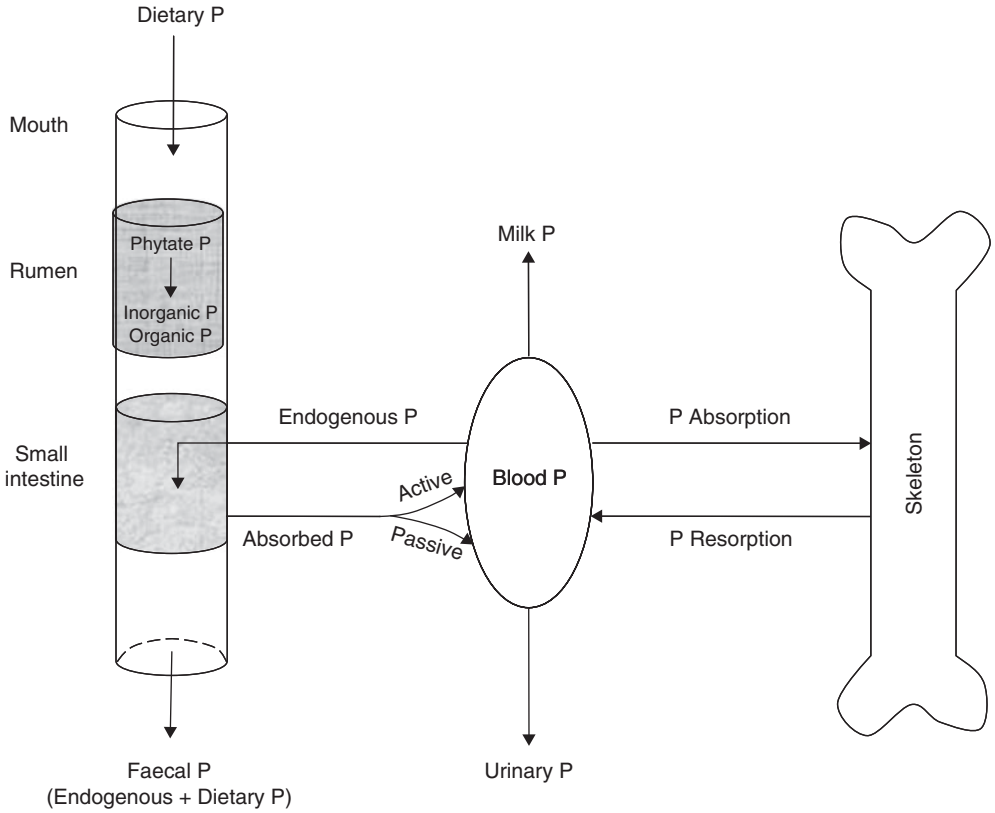

Fig. 7.2. Schematic representation of P metabolism in the ruminant (based on Pond *et al.* 2005).

tissue impulses, maintenance of the integrity of the plasma membrane, excitation of skeletal and cardiac muscle contraction, activation of the blood clotting mechanism, and as a component of milk. The extracellular ionized Ca^{2+} concentration is maintained at about 1 mM, representing about 0.1% of the total body calcium of mammals (Brown *et al*., 1995; NRC, 2001). The skeleton provides a massive reservoir of calcium and phosphate ions for use when the diet is deficient in these ions, or when the intestinal absorption and renal recycling of these ions is inadequate to maintain the extracellular Ca^{2+} and PO_4^- concentrations (Aurbach *et al*., 1985; Stewart and Broadus, 1987).

Normal cellular and nervous system electric potential and conductivity function in mammals is dependent on the nearly constant physiological concentrations of the extracellular monovalent and divalent cations and functional anions (Brown, 1991). Parathyroid hormone (PTH) and the physiologically active form of vitamin D ($1,25(OH)_2D_3$) function metabolically to control Ca and P metabolism and maintain a total plasma (extracellular fluid) Ca^{2+} concentration of 2.2–2.5 mM (9–10 mg/dl or 4.4–5 mEq/l) in adult mammals (Hurwitz, 1996;

NRC, 2001; Hoenderop *et al*., 2005), which is the physiological concentration required for normal bone mineralization and to prevent the occurrence of tetany (uncontrolled muscular contraction) that occurs when the plasma Ca concentration drops to about 5 or 6 mg/dl (Pond *et al*., 2005; DeLuca, 2008). For the mammalian species, the total plasma Ca concentration of about 2.5 mM may be broken down into the following components. Ionic, soluble Ca^{2+} comprises about one-half (1.0 to 1.3 mM) of the total plasma Ca, with small amounts of Ca^{2+} forming complexes with phosphate, citrate, and other anions (about 0.16 mM). The remaining plasma Ca is protein-bound (about 1.14 mM), most of which is present as plasma albumin (0.34 to 0.84 mM) (Walser, 1961; Stewart and Broadus, 1987; Hurwitz, 1996). Hypocalcaemia may occur when body Ca losses exceed absorption and retention, whereas hypercalcaemia may occur with excessive intakes of vitamin D, resulting in soft tissue calcification (DeLuca, 2008).

Physiological regulation of the Ca^{2+} concentration in plasma and extracellular fluid is accomplished through the action of closely controlled complex feedback mechanisms involving three organ systems that function together: the small intestine, bone and kidney. The only route by which the body may acquire Ca^{2+} is by net transport through the intestinal epithelium. Endogenous Ca is removed from the plasma during milk production, bone growth and remodelling, and in the production of digestive secretions, sweat and urine (NRC, 2001). When the plasma Ca^{2+} concentration drops below the required physiological concentration and intestinal absorption is not adequate to maintain Ca^{2+} homeostasis, bone serves as the major reservoir of Ca^{2+} by returning Ca^{2+} to the plasma to maintain the physiological plasma Ca^{2+} concentration (Henry and Norman, 1984; Hurwitz, 1996; DeLuca, 2008).

The sensing of the plasma (extracellular) Ca^{2+} concentration is the first step in the feedback mechanism of Ca^{2+} regulation. A specialized Ca^{2+} sensing molecule/receptor on the cell wall surface detects changes in the extracellular Ca^{2+} concentration (Brown, 1991). The Ca^{2+} sensing receptor has been identified in bovine parathyroid, kidney and brain cells, and cloned from bovine parathyroid cells (Brown *et al*., 1993). The association of Ca^{2+} with its receptor stimulates the release of Ca^{2+} both from within cells and from extracellular spaces, possibly by activation of voltage-sensitive Ca^{2+} channels (Muff *et al*., 1988; Brown, 1991; Hoenderop *et al*., 2005).

Total plasma phosphate concentrations, including both inorganic and organic P, normally range from 4 to 8 mg/dl (1.3–2.6 mM) for dairy cattle, with slightly higher values for young, growing cattle (6–8 mg/dl) compared with adult values (4–6 mg/dl). Whole blood contains six to eight times more P than plasma because of the high concentration of P in erythrocytes (NRC, 2001). The intracellular P concentration in cattle is about 25 mM/l (78 mg/dl). The organic P in plasma is mainly a constituent of phospholipids. For inorganic plasma P, about 10% is bound to serum proteins and 50–60% is ionized. The red blood cells contain P in several constituents as inorganic, organic-acid-soluble lipid and RNA forms of P. All body P is in a dynamic state of turnover, with a half-life of about 1.4 h for blood cells and 197 h for brain (Pond *et al*., 2005).

Intracellular calcium regulation

The intracellular Ca^{2+} concentration in resting cells is maintained at about 10–100 nM, or less than 1/10,000 of the extracellular Ca^{2+} concentration. Intracellular Ca^{2+} participates in an intracellular 'signalling system' by functioning as a diffusible 'second messenger' mediating cellular processes that include apoptosis, axonal flow, endocytosis, exocytosis, cellular division, glycogen metabolism, hormone secretion, immune response, inflammation and heart and muscle contraction (Brown, 1991; Bootman and Berridge, 1995). Calcium can facilitate these intracellular functions after binding with calmodulin, an intracellular Ca^{2+}-binding protein in all eukaryotic cells that functions as a regulator of Ca^{2+}-dependent enzymes and target proteins (Means and Dedman, 1980; Chin and Means, 2000). Hormones are chemicals produced by cells and released into the circulation to control and regulate the activity of specific cells or organs, whereas intracellular Ca^{2+} and nucleotides regulate intracellular organelles. The cellular concentration of Ca^{2+} is controlled by a complex series of transport mechanisms, which includes channels and pumps and other mechanisms that regulate the transport of Ca^{2+} both into and out of the cell and between the cellular organelles (Bootman and Berridge, 1995). Therefore, within activated cells, the free Ca^{2+} concentration functions as a second messenger by conveying information from the surface of the cell to the interior of the cell. Intracellular Ca^{2+} may increase ten- to 100-fold as a result of the uptake of extracellular Ca^{2+} or by the release of Ca^{2+} from sources within the cell such as the Ca^{2+} coupling between endoplasmic reticulum and mitochondria (Linn, 2000; Spät *et al*., 2008).

Hormone regulation of calcium and phosphorus metabolism

In a functional feedback regulating system, a quantitative relationship exists between the secretion rate of the regulatory hormone and the controlled entity, plasma Ca^{2+} or PO_4^- concentration, with the concentration of hormone secreted being proportional to the increase or decrease required in the Ca^{2+} concentration (DeLuca, 2008).

Parathyroid hormone

When plasma Ca^{2+} falls below the physiological threshold concentration, the parathyroid gland releases PTH, the peptide hormone that responds to acute, short term (minutes to hours) perturbations in Ca^{2+} concentrations. In situations of sustained low Ca^{2+} concentrations, PTH stimulates the conversion of vitamin D_3 to the steroid hormone $1,25(OH)_2D_3$, the active form of vitamin D, which functions to increase the intestinal absorption of Ca^{2+} and the deposition of Ca^{2+} in bone, both beneficial for increasing the overall body Ca status (Hoenderop *et al*., 2005; Schröder and Breves, 2007). Another action of PTH is to enhance the reabsorption of Ca^{2+} from the renal tubules. In normal animals, a chronic deficiency of Ca during growth or reproduction may result in parathyroid gland hypertrophy and hyperplasia, whereas a chronic excess of Ca in the circulation results in parathyroid gland involution, most likely by apoptosis (Parfitt, 1994).

The action of PTH is initiated by the binding of PTH to specific membrane receptors in the target organs of kidney and bone. The binding of PTH to the receptor causes a change in the conformation of the receptor that allows it to interact with the heterotrimeric G protein in the cell membrane (Strader *et al*., 1994). The amino acid sequence of PTH has been determined for the bovine (Brewer and Ronan, 1969), the pig (Sauer *et al*., 1974) and the chicken (Khosla *et al*., 1988; Russell and Sherwood, 1989). An analogue of PTH, parathyroid hormone-related protein (PTHrP), is responsible for a mammalian hypercalcaemia malignancy. Both PTH and PTHrP bind to PTH receptors, and have a sequence homology only at the amino terminus. However, PTHrP is not considered to be a calciotropic hormone because of the poor relationship between the circulating concentrations of Ca^{2+} and PTHrP (Chorev and Rosenblatt, 1994; Strewler and Nissenson, 1994).

Vitamin D_3 (cholecalciferol) and vitamin D_2 (ergocalciferol)

Vitamin D_3, also named cholecalciferol, is a fat-soluble vitamin that is found almost exclusively in animals and not in plants. Vitamin D_3 does not have functional biological activity until it is converted metabolically to $1,25(OH)_2D_3$, also named calcitriol, a metabolite that is classified as a secosteroid hormone because of its functional roles in the absorption of Ca and P in the intestine, resorption of Ca^{2+} and P in the kidney and mobilization or accumulation of Ca^{2+} and P in bone (Henry and Norman, 1984; Lee *et al*., 1990; Bouillon *et al*., 1995). In addition, $1,25(OH)_2D_3$ has been shown to have a direct effect on bone tissue growth by regulating the differentiation and proliferation of the cellular elements in bone, as well as in many other tissues, including the immune system, skin (epithelial tissue), some cancerous tissues and pancreatic β-cells (Pols *et al*., 1990; Lee *et al*., 1994). Analogues of $1,25(OH)_2D_3$ also have a role in the control of the cell cycle (Godyn *et al*., 1994). The dihydroxylated vitamin D_3 metabolites are classified as 'hormones' because they fit the established criteria (Schnoes and DeLuca, 1980; DeLuca, 2008).

When 7-dehydrocholesterol, a provitamin D in skin, is exposed to ultraviolet irradiation (sunlight is the natural source of UV irradiation), the provitamin D is converted to provitamin D_3 by a temperature-dependent isomerization (Webb and Holick, 1988; Holick, 1989). Because of a low solubility in water, vitamin D requires a specific α_1-globulin-binding protein for transport in plasma. Vitamin D may be metabolized in liver or stored in adipose tissue where it may be released slowly over time. In the liver, 25-hydroxyvitamin D ($25(OH)D_3$) is synthesized from vitamin D by hydroxylation at position 25. Hydroxylation, which also increases the water solubility of vitamin D, is inhibited by adequate circulating concentrations of vitamin D or $25(OH)D_3$, a feedback mechanism to prevent vitamin D toxicity (Blunt *et al*., 1968; Omdahl and DeLuca, 1973; DeLuca, 2008). The circulating $25(OH)D_3$ is further hydroxylated in the proximal renal tubule cells of the kidney to produce $1,25(OH)_2D_3$ (activated vitamin D_3 or calcitriol), the most potent metabolic form, which fulfils all of the essential metabolic functions of vitamin D_3 for increasing Ca^{2+} absorption and bone growth (Akiba *et al*., 1980; Brommage and DeLuca, 1985; Jones *et al*., 1998). Calcitriol regulates Ca^{2+} absorption by regulating the synthesis of calbindin, a specialized

Ca^{2+}-binding protein found in the cell cytoplasm of mammalian intestine and kidney, which increases Ca^{2+} absorption by shuttling Ca^{2+} across the cell, and also prevents the occurrence of toxic intracellular Ca^{2+} concentrations by sequestering Ca^{2+} in epithelial cells (Christakos *et al*., 1992; Feher *et al*., 1992; Chard *et al*., 1993).

Deficiencies in either Ca or P trigger increases in calcitriol production in mammals, although a greater response in calcitriol production may occur with a Ca deficiency than a P deficiency, as young swine fed a Ca-deficient diet had a fourfold increase in calcitriol production compared with a two- to threefold increase in calcitriol production when fed a P-deficient diet (Fox and Ross, 1985). Of the other vitamin D metabolites produced in the kidney, $24,25(OH)_2D_3$ is the most abundant in the circulation, although the effectiveness and potency of $24,25(OH)_2D_3$ are much less than those of $1,25(OH)_2D_3$ (Holick *et al*., 1971; Norman *et al*., 1971).

Receptors for $1,25(OH)_2D_3$ have been isolated from the cellular components of the tissues involved in the Ca^{2+} control system, which include the mucosal epithelial cells from intestine and kidney, cartilage cells, osteocytes and osteoblasts and many other tissues not associated with Ca^{2+} control (Haussler, 1986; Boivin *et al*., 1987; Jones *et al*., 1998). The number of receptors for $1,25(OH)_2D_3$ in intestinal and bone tissue is higher in young animals than in adult animals, indicating that receptor numbers decline with advancing age (Horst *et al*., 1990). Receptors for $1,25(OH)_2D_3$ have also been found on the basolateral membrane of chicken intestinal epithelium (Baran *et al*., 1994; Nemere, 1995).

Vitamin D_2, also named ergocalciferol, is another form of vitamin D, which is produced in plants, including yeast. When exposed to ultraviolet radiation, vitamin D_2 undergoes the metabolic changes required to become an active form of vitamin D, similar to vitamin D_3. Vitamin D_2 and vitamin D_3 function equally well in many mammalian species (NRC, 1998, 2001). However, pigs discriminate between vitamin D_2 and vitamin D_3 metabolically (Horst *et al*., 1982), and vitamin D_2 has little to no activity (nutritional value) for avian species and some New World primates (Massengale and Nussmeier, 1930; NRC, 1994). The efficiency with which Vitamin D_3 is absorbed from feed by poultry is about 60–70% (Bar *et al*., 1980).

Calcitonin

When the plasma Ca^{2+} concentration exceeds the physiological threshold, C cells in the thyroid gland release calcitonin (CT), a peptide hormone that lowers plasma Ca^{2+}. Calcitonin has been isolated and characterized in bovine, porcine and avian species (Brewer and Ronan, 1969; Nieto *et al*., 1973; Sauer *et al*., 1974). The binding of CT to receptors has been shown in the kidney and in the osteoclasts of bone (Marx *et al*., 1973; Nicholson *et al*., 1986). The CT receptor is a glycoprotein similar to the receptors for PTH and the other peptide hormones (Hurwitz, 1996). Analogous to the control of PTH secretion, the secretion of CT is controlled by cAMP as the second messenger, following activation of the adenylate cyclase system by β-agonists, glucagon and pancreozymin-cholecystokinin and other structurally related digestive hormones (Care *et al*., 1970, 1971).

The physiological action of CT is to lower plasma Ca^{2+} by decreasing or preventing bone resorption. In the kidney, CT stimulates 25-hydroxyvitamin D_3-1-hydroxylase production, which is also associated with the production of cAMP. The overall physiological importance of CT, however, may be less than that of PTH in maintaining Ca homeostasis in mammals (Hurwitz, 1996). Similar to what occurs with PTH and other peptide hormones, continuous exposure of the tissues to CT results in down-regulation of receptor binding (Obie and Cooper, 1979).

Organ systems involved in calcium and phosphorus metabolism

Intestinal absorption of calcium and phosphorus

Activated vitamin D_3 increases the transport of Ca^{2+} across the intestinal mucosa, primarily by transmembrane diffusion facilitated by a positive electrochemical gradient in the presence of an adequate supply of ionic Ca^{2+} (about 6 mM or greater) in the digestive fluids (Bronner, 1987). In non-ruminants, up to 50% of the dietary Ca^{2+} is absorbed by passive transport, with active transport in situations of greater plasma Ca^{2+} deficiency. For ruminants, the dilution effects of the rumen suggest that active transport of Ca^{2+} is most likely the major route of Ca^{2+} absorption in mature ruminants (NRC, 2001). Absorption of Ca^{2+} occurs by diffusion directly through the mucosal cells, called transcellular absorption, and by diffusion through the intercellular junctions between the cells, called paracellular absorption (Hurwitz and Bar, 1968, 1972; Fullmer, 1992; Hoenderop *et al*., 2005). Calmodulin, a calcium-binding protein expressed in all eukaryotic cells, functions as a Ca^{2+} channel protein to transport Ca^{2+} away from the brush border of intestinal cells (Kaune *et al*., 1992). Calbindin-D_{9k}, a vitamin D-induced Ca^{2+}-binding protein in the intestine and kidney of mammals, transports Ca^{2+} across the intestinal cell from the apical (lumen) side to the basolateral side. A similar Ca^{2+}-binding protein, calbindin-D_{28k}, has the same function in poultry (Bronner *et al*., 1986). Additional research found that, when 1,25$(OH)_2D_3$ is present in mammalian small intestine, calbindin-D_{9k} is not required for Ca^{2+} absorption (Kutuzova *et al*., 2006; Akhter *et al*., 2007). Calbindin-D_{9k} also functions as a buffer for Ca^{2+} ions in pig duodenal enterocytes (Schröder *et al*., 1996). A saturable ATP-activated Ca^{2+} pump may be stimulated by 1,25$(OH)_2D_3$ to actively transport Ca^{2+} uphill (against a concentration gradient) across the basolateral membrane (Wasserman *et al*., 1992; Hoenderop *et al*., 2005). Genes regulating intestinal Ca^{2+} absorption are all up-regulated in response to 1,25$(OH)_2D_3$ (Kutuzova and DeLuca, 2004).

Bone function in calcium and phosphorus metabolism

The primary mineral salt in bone occurs as hydroxyapatite crystals, $Ca_{10}(PO_4)_6(OH)_2$, with Ca and P in about a 2:1 ratio, although other less stable calcium phosphates may also be produced (Glimcher, 1992; Merck and Co., Inc., 2006). Trabecular bone is a readily available reservoir of Ca and P for resorption and transfer into the plasma to maintain homeostasis in mammals (Bauer *et al*., 1929; Eklou-Kalonji *et al*., 1999). Bone is a metabolically active tissue, and the

Ca turnover rate in bone varies widely depending on the type of bone, with a half-life (based on ^{45}Ca) ranging from several months in cortical bone to 1 or 2 days in medullary bone (Hurwitz, 1965). In addition to bone marrow, the other five cell types involved in bone growth and remodelling include: chondrocytes, cartilage cells that are found mainly in the growth plate region of bone; osteoblasts, bone-forming cells that originate from mesenchymal stem cells, located in the growth plate and on the periosteal and endosteal surfaces of bone cells; osteocytes, mature bone cells that originate from osteoblasts after the osteoblasts become embedded in osteoid (mature) bone; osteoclasts, bone-resorption cells in the lacunar spaces of bone, spaces that are interconnected with each other, with bone surface cells, and blood capillaries; and periosteum, bone membrane tissue that consists of an outer protective fibrous layer and an inner cellular osteogenic layer. The embryonic skeleton is mainly cartilage and fibrous tissue, which are converted to bone during growth and development. The membranous bones of the head and face develop from fibrous tissue, whereas most of the skeletal bone originates from preformed cartilage, which is converted to bone during growth (Sisson and Grossman, 1962).

Normal bone mass is maintained by a balance between osteoblast and osteoclast activity (Takeda *et al*., 2003; Fu *et al*., 2005). Osteoblasts have receptors for PTH and $1,25(OH)_2D_3$, and these hormones modulate the activity of alkaline phosphatase and the transport characteristics of Ca^{2+} in bone growth and remodelling. The rate of chondrocyte proliferation and conversion to osteoid bone is also enhanced by growth hormone, the somatomedins, including insulin-like growth factor (IGF)-1 and IGF-2, and other growth factors. In addition, both $1,25(OH)_2D_3$ and $24,25(OH)_2D_3$ are required for the differentiation of the various bone cells during normal bone growth (Schwartz *et al*., 1992; Hurwitz, 1996). The organic matrix of bone must be mineralized, primarily with Ca and P, to produce a mature, strong bone. Bone resorption by osteoclasts is controlled by PTH, apparently mediated through other cells that have PTH receptors, such as osteoblasts or chondrocytes, because mammalian osteoclasts lack PTH receptors. The involvement of interleukin-1 in bone resorption also appears to require mediation through osteoblasts. Conversely, calcitonin is a powerful inhibitor of osteoclastic bone resorption in mammals when the plasma Ca^{2+} concentration is above normal (Hurwitz, 1996). Leptin, an adipocyte-derived hormone, is also a strong inhibitor of bone formation through mediation involving the brain and the sympathetic nervous system (Takeda *et al*., 2003; Fu *et al*., 2005).

Kidney function in calcium and phosphorus metabolism

About 96–99% of the Ca^{2+} and 85–90% of the P is reabsorbed during glomerular filtration in the kidney, with about half by transcellular (through the cell) diffusion and half by paracellular (between the cells) diffusion, similar to the absorption process that occurs in the small intestine. In contrast to the transcellular absorption, paracellular absorption of Ca^{2+} and P is dependent on the cellular concentrations of Na^+ and Cl^-. Most of the Ca^{2+} and P are reabsorbed in the proximal convoluted tubule, even though the distal tubule is the main site of hormonal regulation (Kumar, 1995; Hurwitz, 1996). Only about 10% of the Ca^{2+} and P are reabsorbed in the distal tubule (Friedman and Gesek, 1993). In

contrast to the proximal tubule, the Ca^{2+} concentration in the ultrafiltrate passing through the distal convoluted tubule may drop to 0.1 mM, resulting in an uphill (active) Ca^{2+} transport situation. At the distal convoluted tubule, hormone-sensitive transcellular absorption appears to be the primary route of Ca^{2+} and P absorption. The transport of Ca^{2+} is through the apical or basolateral membrane by movement through Ca^{2+} channels. The reabsorption of Ca^{2+} and P at the distal tubule is increased by PTH, and by $1,25(OH)_2D_3$ through an interaction with PTH, and Ca^{2+} reabsorption is decreased by calcitonin. Receptors for $1,25(OH)_2D_3$ are found along the entire tubular system of the nephron (Friedman and Gesek, 1993; Gesek and Friedman, 1993). When plasma phosphate becomes elevated, vitamin D functions to increase the excretion of phosphate in the kidney to correct the plasma hyperphosphataemia, which suppresses plasma Ca^{2+}. High phosphate also results in the release of PTH in an attempt to maintain physiological plasma concentrations of Ca^{2+} (DeLuca, 2008).

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8 Phosphorus and Calcium Requirements of Ruminants

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Introduction

Pfeffer *et al*. (2005) extensively reviewed P metabolism in ruminants and requirements of cattle. In this chapter, some of the information is updated and expanded to include Ca requirements. Phosphorus and calcium requirements in small ruminants are also included in this chapter. The chapter reflects the tremendous amount of work conducted on P compared with Ca; therefore, more emphasis is given to P requirements.

Increased specialization and concentration of livestock and crop production have led to the net export of nutrients from major crop-producing areas of the country to areas with a high concentration of animal agriculture. Livestock utilize P inefficiently, excreting 60 to 80% of that consumed. Therefore, the majority of P brought on to the farm in feed stays on the farm, rather than being exported in meat or milk.

Animal manure is typically land-applied to supply nutrients for crop growth, but N and P are in imbalance in manure relative to crop needs. Land-application of manure to meet the N needs of the crop results in the over-application and accumulation of P in soils. Historically, P contamination of surface water was thought to be associated primarily with erosion. As application of P in excess of crop requirements continues, however, soil becomes saturated and runoff of P can occur independently of erosion (Daniel *et al*., 1992).

Areas facing the dilemma of an economically important livestock industry concentrated in an environmentally sensitive area have few options. If agricultural practices continue as they have in the past, continued damage to water resources and a loss of fishing and recreational activity are almost inevitable. If agricultural productivity is reduced, however, the maintenance of a stable farm economy, a viable rural economy and a reliable domestic food supply is seriously threatened. Practices that reduce P losses from farms without impairing profitability must be developed and implemented. Improved understanding of P

digestion, absorption and metabolism in livestock will improve efficiency of P utilization, reducing P excretion and minimizing the imbalance of N and P in manure (Knowlton *et al*., 2004).

Role of Animal Agriculture

Concentrated animal agriculture has been identified as a significant source of P contamination of surface water in the United States (median contribution = 7 to 48% of total P loads, depending on watershed; Smith and Alexander, 2000). These losses were calculated with a model using measured stream water quality data and spatial data on sources, landscape characteristics and stream properties. The link between animal numbers, manure application to a limited land area and P contamination of surface water was also demonstrated in the Lake Okeechobee watershed in Florida. From 1973 to 1988, P concentration in the water of Lake Okeechobee in Florida increased by 250% (Negahban *et al*., 1993). During this same time period, dairy cow numbers in the three counties surrounding the lake increased by more than 900 cows per year (Boggess *et al*., 1997), and dairies were identified as the source of about 40% of the P load to the lake (Negahban *et al*., 1993). The appearance of lake-wide algae blooms led to the imposition of stringent regulations designed to reduce agricultural runoff.

Increasing Regulatory Pressure

Increasing public concern with water quality and increased awareness of the impact of concentrated livestock production have led to the development and implementation of increasingly stringent environmental regulations. Greater pressure on states from the federal government in the last decade to enforce federal clean water regulations has significantly increased the level of regulatory pressure felt by farmers in the USA. Similarly, in Manitoba (Canada), the provincial government has implemented a bill that permanently bans building or expanding animal facilities along the Red River Valley, south-eastern Manitoba and the interlake region (between Lake Winnipeg and Lake Manitoba). These regulations were implemented to reduce P loading and contamination of water bodies (MLMMI, 2009).

Limiting manure application to the P needs of the crop is one way to avoid continued accumulation of P in soil and to minimize potential P runoff and contamination of surface water. Regulations limiting manure application to the P needs of the crop are explicitly allowed in the recently finalized federal Concentrated Animal Feeding Operation regulations to address water pollution in the USA (EPA, 2001). These federal regulations call for site-specific decisions on whether N- or P-based manure application limits are needed to protect water quality, and P-based limits are now enforced in several states (e.g. Michigan, Maryland, Virginia and Florida). Also, some federal cost-share funding is now being linked to the development and implementation of P-based nutrient management plans. Phosphorus-based nutrient management regulations dramatically

increase the amount of land required to dispose of manure, and will have a severe, detrimental effect on the agricultural economy in areas of intensive animal agriculture. In Canada, Quebec and Manitoba have introduced regulations that limit manure application. For example, Manitoba Conservation (2009) has recently implemented a Livestock Manure and Mortalities Management Regulation, which requires producers to test P content of soil and determine the amount of manure that can be applied based on soil test P.

Fundamental Basis for Phosphorus and Calcium Requirement in Ruminants

Phosphorus is an essential nutrient involved with bone development, growth and productivity, and with most metabolic processes of the body. Phosphorus and Ca are the two most plentiful minerals in ruminants (Kebreab and Vitti, 2005). Phosphorus constitutes 1% of the total body weight (BW), 80% of which is found in the bones. The remaining 20% is distributed in body cells and is involved in maintaining the structural integrity of cells and in intracellular energy and protein metabolism. Most of the Ca in ruminants (99%) is found in bones and teeth; the remaining 1% is distributed in various soft tissues (McDowell, 1992).

The P requirement in ruminants is for potentially available (absorbable) P in the diet. Phosphorus is consumed by the animal as organic (phytates, phospholipids and phosphoproteins) and inorganic P (mono-, di- and triphosphates). Soluble and some water-insoluble forms are dissolved by digestive juices in the rumen. The availability of P in the diet has been the subject of many investigations and is discussed in detail in Chapter 6. 'True absorption' coefficients have been used to describe the amount of dietary P absorbed but do not reflect the potentially available dietary P because true absorption coefficients decline with increasing P intake.

Saliva can contribute up to 50% of P entering the rumen, plays a significant role as a buffer and is important as a nutrient source for rumen microbes (Care, 1994). For example, Kebreab *et al*. (2005) observed that, on average, salivary P inputs represented 45% of the total P flow at the duodenum of lactating dairy cows fed grass silage-based diets. Significant variation in P concentration in saliva of ruminants has been observed (Pfeffer *et al*., 2005). Salivary P content does not affect salivary flow. Dijkstra *et al*. (1992) developed an equation that relates salivation rate to DMI and NDF content of the diet and found that estimates from the model were within 10% of those observed by Valk (2002).

Phosphorus is an important component of the cell membrane and is essential for microbial growth. Czerkawski (1976) estimated P contents of protozoa, large and small bacteria in the rumen to be 1.38, 1.33 and 1.88 g/100 g of polysaccharide-free microbial DM, respectively. These are close to Durand and Kawashima's (1980) estimate of 1.44% for an average P content of rumen bacteria. However, these are at the lower end of concentrations estimated by Hungate (1966), who reported that rumen microbe cells contain 20 to 60 g P/kg DM. Microbial P is present as nucleic acids (80%), phospholipids (10%) and

other compounds. High P concentrations occur in the rumen contents, ranging from 200 to 600 mg/l (Witt and Owens, 1983).

Microbial P constitutes a major proportion of P entering the small intestine. Pancreatic ribonuclease breaks down microbial RNA and P is released (Barnard, 1969). It is generally accepted that the upper small intestine, where the pH of the digesta is acidic, is the major site for P absorption (Breves and Schröder, 1991). Chapter 7 discusses in detail the processes involved in absorption of P in ruminants. The large intestine of ruminants also has the capacity to absorb significant quantities of P (Milton and Ternouth, 1985); however, most of it is present as insoluble or nucleic acid P (Poppi and Ternouth, 1979). Yano *et al*. (1991) concluded that in sheep, little absorption or secretion of P appears to occur in either the rumen or the large intestine.

Requirement for P also includes incorporation into milk, metabolic activity in tissues and storage in bone and tissues. Besides its structural function, bone represents a reserve of P. According to Sevilla (1985), when P deficiency occurs, more than 40% of the animal's requirement can be supplied by bone resorption. Milk P output is directly related to milk yield as milk P concentration is constant (0.9 g/kg of milk; Fox and McSweeney, 1998; NRC, 2001). Phosphorus in soft tissues can be present as lecithin, cephalin and sphingomyelin and in blood as phospholipids (Cohen, 1975). Blood is the central pool of readily available minerals and contains 350 to 450 mg P/l, mostly present in the cells. Plasma P is present mainly as organic compounds and the remainder is in inorganic form, as PO_4^{3-}, HPO_4^{2-} and $H_2PO_4^-$ (Georgievskii, 1982). Normal blood P is between 40 and 90 mg P/l in sheep; values lower than 40 mg are indicative of deficiency. In cattle, P intake varying from 27.1 to 62.5 mg P/kg LW resulted in a plasma P of 47 to 77 mg/l (Underwood and Suttle, 1999).

Absorption or resorption of P is also associated with requirement for Ca because the two minerals are closely related. Phosphorus is present in bone as hydroxyapatite molecules, tricalcium phosphate and magnesium phosphate. The Ca:P ratio in bone is almost constant at 2:1. The effect of an unbalanced Ca:P ratio in the ruminant diet is discussed in Chapter 7.

Calculation of Phosphorus and Calcium Requirements

Dairy cattle

The National Research Council (NRC) periodically reviews and summarizes the nutrient requirements of various species, and issues publications listing these requirements. In the current dairy NRC, the P requirement is described using a factorial approach (NRC, 2001). The absorbed P requirements to support maintenance, growth, pregnancy and lactation are calculated and summed, and then adjusted based on the estimated bioavailability of P in various feedstuffs and supplemental sources to calculate the amount of P that must be fed. Other national research organizations such as the British (AFRC, 1991) and French (INRA, 1989) also used the factorial approach to estimate P requirements.

Table 8.1. Phosphorus requirements for Holstein cows (600 kg BW) with varying DMI and milk yield (NRC, 2001).

	Milk yield, kg/day						Milk yield, kg/day					
	30	32	34	36	38	40	30	32	34	36	38	40
DMI, kg/day	Absorbed P requirement, g/day						Dietary P requirement, % of diet DM[a]					
21.8	49	51	52	54	56	58	0.35	0.36	0.37	0.39	0.40	0.41
22.5	49	51	53	55	57	58	0.33	0.34	0.35	0.37	0.38	0.39
23.2	50	52	54	56	57	59	0.32	0.34	0.35	0.36	0.37	0.38
23.9	51	53	54	56	58	60	0.32	0.33	0.34	0.35	0.36	0.38
24.6	52	53	55	57	59	61	0.31	0.32	0.34	0.35	0.36	0.37
25.3	52	54	56	58	60	61	0.31	0.32	0.33	0.34	0.35	0.36

[a]Shaded cells indicate dietary P concentrations based on NRC predicted DMI for the specified rate of milk yield.

However, calculations relating to maintenance requirements especially vary among the various national organizations.

Table 8.1 describes the total absorbed P requirement for non-pregnant, mature, lactating Holstein cows of varying milk yields and dry matter intake (DMI), and the dietary P concentration required for these non-pregnant mature cows (NRC, 2001). The P availability used to calculate this dietary requirement is from the sample lactating cow diet included in the software distributed with the publication.

Estimation of maintenance requirement

Fundamental research findings to estimate the P maintenance requirement are presented by Pfeffer *et al*. (2005). Typically the maintenance requirement is about one-third to one-half of the dairy cow's total dietary P requirement. Therefore, accurate estimation of the P maintenance requirement is important to optimize the cow's P economy and overall performance, and to reduce P excretion.

The maintenance requirement for absorbed P is defined as the inevitable (unavoidable) loss of P in faeces (by far the largest portion) plus the generally small amount of endogenous urinary P when the healthy animal is fed close to its absorbed (true) P requirement (NRC, 2001; Pfeffer *et al*., 2005). Roughly two-thirds of the inevitable faecal P loss is assumed to be associated with microbial debris from the digestive tract (Kincaid and Rodehutscord, 2005).

Approaches to estimating, and the values reported for, the P requirement for maintenance of dairy cows have varied (ARC, 1980; INRA, 1989; NRC, 1989, 2001; Pfeffer *et al*., 2005). Previously, the daily P requirement for maintenance was expressed as mg/kg BW (ARC, 1980; INRA, 1989; NRC, 1989). The ARC (1980) suggested that the net or absorbed P requirement for maintenance was about 12 mg of P/kg BW/day. This estimate was based on experimental data of faecal P excretion extrapolated to a P intake of zero. Based on the ARC (1980) approach, the NRC (1989) estimated the total dietary requirement of P for

maintenance as: g/day = (0.0143 × BW (kg)) ÷ 0.50, where the denominator is an estimate of the absorption coefficient for P in the overall diet. The Institut National de la Recherche Agronomique (INRA, 1989) stated that the P maintenance requirement of 12 mg of P/kg BW/day reported by ARC (1980) was too low and suggested that the P maintenance requirement was probably between 23 and 32 mg P/kg BW/day for cows yielding between 5 and 50 kg of milk/day. Also, the AFRC (1991) estimated the P maintenance requirement as: (g/day) = 0.693 × DMI (kg/day) – 0.06; in this case the requirement was equal to the daily inevitable faecal P excretion, but did not account for endogenous urinary P. Also, the equation was developed from data with sheep; this likely affects its relevance to dairy cattle.

Subsequently, Spiekers *et al*. (1993) estimated inevitable faecal P loss of high versus low-yielding cows (five per group) with similar BW but different DMI (17 (high) versus 11 (low) kg/cow/day) and milk yield (21 (high) versus 10 (low) kg/cow/day). Both groups were fed the same diet with 0.21% P, dry basis. Phosphorus intakes differed (37 versus 22 g/cow/day for high and low, respectively). Inevitable faecal P loss (the faecal component of the maintenance requirement for P) as a function of feed intake rate was 1.20 and 1.22 g of P/kg of dietary DM for the high and low intake groups, respectively. The theoretical assumptions of this estimation approach are that cows are fed very near their total true (absorbed) P requirement (g/day) and that the absorption coefficient of dietary P approaches 100%. However, the productivity of the cows in this study was much less than that of many modern dairy cows.

Utilizing the same general approach, the NRC (2001) subcommittee set the inevitable faecal component of the absorbed P requirement for maintenance of non-lactating pregnant and lactating cows at 0.8 g/kg DMI, plus a small amount for endogenous urinary P (0.002 g/kg BW). This somewhat conservative approach, with the assumption of a dietary P absorption coefficient of 0.80, would yield a prediction of 1.0 g of dietary P/kg DMI as the inevitable faecal P fraction of the total dietary maintenance requirement.

Obviously, the actual dietary P requirement to meet the inevitable faecal P loss is dependent upon the absorption coefficient of P from that particular diet. Recent summaries of much of the world literature on ruminal availability and whole-tract absorbability (e.g. absorption coefficients) of P in cattle strongly suggest that values of each are commonly greater than 90% from ordinary feedstuffs when dietary P is fed near the animal's true or net requirement for P (Kincaid and Rodehutscord, 2005; Pfeffer *et al*., 2005).

Presumably correct conceptually, NRC (2001) proposed absorption coefficient estimates of P for some feeds drawing on the available literature. However, estimation of the dietary P requirement remains imperfect, in part, because of unknown and perhaps variable absorption coefficients among typical feed ingredients. None the less, the inevitable faecal P loss is the largest portion of the absorbed and dietary P requirement for maintenance.

Results of recent research (Myers and Beede, 2008) compared earlier estimates (Spiekers *et al*., 1993) of inevitable faecal P excretion and the P maintenance requirement using higher-yielding, multiparous, lactating Holstein cows over a much wider range of DMI (11.3 to 25.1 kg/cow/day) and milk

production (25.3 to 47.3 ± 1.23 kg/cow/day). All cows were fed the same low-P diet (0.26% P, dry basis). Phosphorus balances of cows were not different from zero and were unaffected by the rate of DMI. Average daily, total, inevitable, faecal P excretion ranged from 15.3 to 26.3 g/cow/day. Inevitable faecal P excretion ranged from 1.36 to 1.04 g/kg DMI from lowest to highest feed intake. The regression equation to estimate inevitable faecal P excretion (IFPE) across the range of DMI was:

$$\text{IFPE (g/day)} = (0.85 \pm 0.070\ \text{(g/day)}) \times \text{DMI (kg/day)} + (5.30 \pm 1.224\ \text{(g/day)}); (R^2 = 0.90; P < 0.01)$$

This equation estimates the inevitable faecal P component of the total P maintenance requirement of lactating Holstein cows.

This general approach seems valid. However, the practical dietary requirement of P for maintenance will be influenced by the absorption coefficient for P applied in that factorial estimation. For animals fed close to actual requirements, the absorption coefficient likely exceeds 90%.

Furthermore, Myers and Beede (2008) suggested that the P in microbial cell debris in faeces should be counted as part of inevitable faecal P and thus part of the P maintenance requirement. From the literature, the P in the microbial fraction is estimated to be about two-thirds of the total faecal P loss (Kincaid and Rodehutscord, 2005). Whereas counting this P in microbial cell debris seems to reach beyond the classic definition for absorbed P for maintenance, which includes only P that has been absorbed, the approach fits with the practicality of meeting the cow's need for normal P maintenance. The microbial population in the digestive tract of ruminants is indispensable for normal digestion of dietary organic matter, for synthesis of microbial protein and for animal survival; these processes must be maintained. The P requirement of the microbial population in the digestive tract is not taken into account in any other way in current requirement estimates (AFRC, 1991; NRC, 2001).

Phosphorus needs for late pregnancy and effects in the periparturient period

The P requirement for gestation of cattle has not been studied extensively. However, it is assumed to be relatively small during the first two trimesters and then to increase significantly during the last ~100 days as conceptus mass increases greatly. The overall requirement for pregnancy is still small compared with that for maintenance and lactation.

The most recent (NRC, 2001) estimates of dietary P requirements and ration recommendations for late pregnant, non-lactating dairy cows are less than those of earlier editions (NRC, 1978, 1989). This is largely because of changes in the way the maintenance requirement for P is estimated (discussed above) and changes in estimates of the absorption coefficients for dietary P.

Until recently, only two studies compared the effects of different prepartum dietary P concentrations on cows during the periparturient period (Kichura *et al*., 1982; Barton *et al*., 1987). Barton *et al*. (1987) examined dietary P concentrations that were all well above the current estimated requirement. Kichura *et al*. (1982) used limit-fed Jersey cows fed semi-purified diets. Thus, these studies

were not with contemporary Holstein cows with greater metabolic demands and genetic potential for milk production.

Peterson *et al*. (2005) compared the effects of different prepartum dietary P concentrations on periparturient metabolism and lactational performance of multiparous Holstein cows. Cows were fed 0.21, 0.31 or 0.44% P (dry basis) for 4 weeks before expected calving. After parturition, all cows were fed a common lactation diet (0.40% P). In the prepartum period, cows fed 0.21% P had lower blood serum P concentrations compared with cows fed 0.31 or 0.44% P. However, serum P concentrations of all cows were within the normal range (4 to 8 mg/dl) until the day of calving when average concentrations dropped below 4 mg/dl. From 3 to 14 days postpartum, serum P of cows fed 0.21% P was greater than that of cows fed 0.31 or 0.44% P. No cows presented or were treated for clinical hypophosphataemia in the periparturient period. Total serum Ca was lower before calving through 2 days postpartum for cows fed 0.44% P compared with those fed 0.21 or 0.31%. Prepartum dietary P treatments did not alter concentrations of blood osteocalcin, hydroxyproline and deoxypyridinoline, indicators of bone metabolism, or concentrations of parathyroid hormone or 1,25-dihydroxyvitamin D_3. Energy-corrected milk yield and milk composition (first 28 days of lactation) were not affected by prepartum dietary P concentrations. It was concluded that feeding 0.21% P (34 g of P/cow/day) prepartum is adequate for periparturient multiparous Holstein cows with high metabolic demands and genetic potential for milk production. No adverse effects on periparturient health, DMI or 28-day lactation performance resulted.

An important point to emphasize is that, like other nutrients, the requirement of the animal for P is for absorbed quantities of P, not dietary concentrations. For convenience in balancing rations, P requirements are commonly expressed as a percentage of dietary DM. The actual dietary concentration needed to yield the required quantity of absorbed P, however, varies with DMI and dietary ingredients composing the ration. For instance, the current NRC requirement for a 600 kg dairy cow producing 36 kg/day of milk is ~56 g of absorbed P per day (Table 8.1). The dietary P concentration required in the diet DM for this cow is 0.37% to 0.33%, as DMI varies from 20 to 25 kg/day.

The Ca requirement of a 600 kg lactating cow producing 20 kg/day, which allows for loss, maintenance or gain in BW, has been estimated to be 51–70 g/day depending on feed quality (AFRC, 1991). Ca requirement for a similar lactating cow was estimated to be about 52 g absorbable Ca/day (NRC, 2001), which is at the lower end of the AFRC (1991) estimate.

Beef cattle

The P requirements for beef cattle in the latest edition of NRC (1996) are calculated using the factorial method, and are based on current BW and rate of protein gain, milk production and fetal weight in the last 3 months of pregnancy for cows and heifers. The maintenance requirement is considered to be 16 mg P/kg BW, reflecting endogenous faecal P loss. Phosphorus requirements for growth are calculated as 3.9 g P/100 g of protein gain, based on data published

Table 8.2. Phosphorus requirements for growing and finishing Angus cattle of varying BW and ADG (NRC, 1996).

	Body weight, kg					
	200	250	300	350	400	450
ADG, kg/day	Dietary P requirement, g/day					
0.5	11	11	12	12	14	15
1.0	16	16	16	16	18	18
1.5	21	21	20	20	21	21
2.0	26	25	25	24	25	24
2.5	31	30	29	27	27	26

in 1950. Availability of feed P is assumed to be 68% from all sources. As an example, the dietary P requirements (g/day) for growing and finishing Angus steers are presented in Table 8.2. The dietary P concentration needed to meet these requirements varies widely with DMI, breed, BW, growth rate and physiological state. In lactating beef cows, P needs for milk are similar to those in the dairy NRC, at 0.95 g/kg milk, and fetal P requirements are 7.6 g/kg fetal weight during the last 3 months of gestation.

These published requirements may overestimate the actual P requirements for growing and finishing cattle. Erickson *et al*. (1999) fed 66 cross-bred finishing steers diets containing 0.14, 0.19, 0.24, 0.29 or 0.34% P for 105 days. The measured P intakes ranged from 15.9 g/day to 36.4 g/day; the calculated P requirement for these steers was 22.5 g/day. Although the two low P diets were deficient in P according to the NRC (1996), these authors observed no effect of dietary P on DMI, average daily grain ADG, feed:gain ratio, carcass weight, bone strength, meat tenderness or marbling. They concluded that maize-based diets contain adequate P to meet the requirements of growing and finishing steers without any supplemental P.

The calcium requirement of growing and finishing cattle was estimated to be 19–48 g/day depending on the quality of feed. For pregnant replacement heifers the range of Ca requirement was 21–32 g/day and for beef cows 16.5 to 34.1 g/day depending on time since conception or calving, respectively (NRC, 1996).

Small ruminants

Phosphorus and Ca requirement calculations for sheep and goats are based on a factorial approach and aim to satisfy the needs for maintenance, growth, gestation and production. For instance, AFRC (1991) calculated maintenance requirements for P (P_m, g/day) and Ca (Ca_m) for sheep fed at least 50% forage as:

$$P_m = (0.693 \text{ DMI (kg/day)} - 0.06) \times 1.6$$

$$Ca_m = 0.623 \text{ DMI} + 0.228$$

The P_m equation also applies to cows and goats (AFRC, 1991). However, a meta-analysis by Pfeffer (1989) showed a lower P_m estimation for goats (0.081 + 0.88 DMI). Requirements for P and Ca in goats along with other minerals were reviewed by Meschy (2000), who recommended that estimates obtained for sheep should be used for goats due to paucity of data. Vitti *et al*. (2000) determined P_m in goats using the isotope dilution technique (which is discussed in detail in Chapter 2) and reported it to be 0.61 g P/day, which is even lower than previous estimates.

Growth requirement for goats is calculated to be 5.8 and 9.4 g/kg BW gain, for P and Ca, respectively (Pfeffer *et al*., 1996). In sheep, growth requirements are based on P accretion (P_g, g/kg) in bone and tissue and Ca (Ca_g, g/kg) accretion in bone (AFRC, 1991), which can be calculated as follows:

$$P_g = 1.2 + 3.19\ MBW^{0.28}\ BW^{-0.28}$$

$$Ca_g = 9.83\ MBW^{0.22}\ BW^{-0.22}$$

where MBW is mature BW (kg).

Pregnancy and lactation requirements for P and Ca in sheep proposed by ARC (1980) were adopted by AFRC (1991) due to lack of additional data. The values for requirement of a 40 kg pregnant ewe range from 0.43 to 3.37 g P/day and 0.8 to 3.86 g Ca/day, depending on the quality of feed and time before parturition. In pregnant and lactating goats, P and Ca requirements are to replace mineral transfer to the kid and replace P output in milk. During the gestation period, the net transfer is estimated to be 25–50 g P and 40–90 g Ca (Meschy, 2000). Phosphorus and Ca content of milk varies depending on breed and stage of lactation. On average, goat's milk contains 0.97 and 1.26 g/l P and Ca, respectively (Meschy, 2000).

Reduced Overfeeding to Reduce P Content of Excreta

In all species of livestock, P fed in excess of animal requirements is excreted, making reduced overfeeding a powerful tool to reduce the P content of manure. In dairy cows, several studies indicate a direct link between P intake and P excretion (e.g. Morse *et al*., 1992; Wu *et al*., 2000, 2001; Knowlton *et al*., 2001; Knowlton and Herbein, 2002). A Florida study was among the first to show this link (Morse *et al*., 1992). Twelve cows were fed diets containing one of three concentrations of P (0.30%, 0.41% or 0.56% of dietary DM). Excretion increased linearly with increasing P intake and accounted for nearly all of the difference in P intake with the highest P diet compared with the low P diet.

Overfeeding of dietary P is common in the field. In the past, P was often fed to dairy cattle 20 to 40% in excess of published requirements (Shaver and Howard, 1995; Sink *et al*., 2000). A survey conducted by Wu (2003) in Pennsylvania indicated that the extent of overfeeding is less now than was indicated in these earlier surveys. In growing beef cattle, Erickson *et al*. (1999) reported an industry average dietary P content of 0.35 to 0.39% of DM, compared with published requirements for growing steers of about 0.20% P. Kebreab *et al*. (2008) conducted a survey in Ontario, Canada, and found that dairy farmers were, on

average, using 0.41% P in the diet. The authors evaluated a dynamic model based on experiments conducted in Ontario (Odongo *et al*., 2007) and reported that reduction of P to NRC (2001) recommended concentrations would save farmers $20 per cow per year. Moreover, excretion of P would be reduced by 1.3 kt/year from Ontario dairy farms according to model calculations (Kebreab *et al*., 2008). Estimates of savings in feed cost assume that the excess dietary P is from purchased mineral sources; if excess P is from inexpensive by-product feeds, reducing dietary P may increase feed costs.

On-farm implementation

Limited work has been published on the impact of reduced overfeeding on herd performance and on-farm nutrient balance. Cerosaletti *et al*. (2004) conducted a study on four dairy farms to identify feeding strategies in commercial dairy herds to reduce manure P and whole-farm P balance. Monthly, lactating cow diets were evaluated and milk production and herd reproductive performance were measured. Manure P content was measured every 6 months. Reduced P diets were implemented in two herds, reducing dietary P intake by 25%. After dietary adjustments in the two herds, faecal P concentrations decreased 33%, with no impact on milk yield. Reducing dietary P content closer to requirements reduced whole-farm P balance by 49%.

Why overfeed?

In dairy cows, the most common explanation for overfeeding of dietary P is the perception that high P diets improve reproductive performance. This perception likely originates from the observation that severe P deficiency impairs reproductive performance in cattle. The original studies that established this belief were primarily with range cattle (Eckles *et al*., 1932; Beeson *et al*., 1941), and the dietary P concentrations necessary to induce this impaired reproductive performance were below 0.20% of the dietary DM for lactating cows. This dietary concentration is far below the concentration found in most feedstuffs in modern lactating cow and beef cattle rations, even without supplementation, and in all of these studies reduced P intake was seriously confounded by marked reduction of intake of energy, protein and other minerals.

Although severe P deficiency may impair reproductive performance, there is no research to suggest a benefit from feeding P to dairy cows in excess of NRC requirements (Brodison *et al*., 1989; Brintrup *et al*., 1993; Wu *et al*., 2000; Lopez *et al*., 2004a, b). A review by researchers in Wisconsin summarized 13 studies with 785 lactating cows fed diets low in P (0.32 to 0.40% P) or high in P (0.39 to 0.61% P; Satter and Wu, 1999). Dietary P had no effect on days to first oestrus, days open, services per conception, days to first AI or pregnancy rate (Table 8.3).

Two other factors that have led farmers to overfeed P are compensation (safety margin) for suspected undetected variation in the P content of feeds, and

Table 8.3. Reproductive performance of lactating cows fed diets low or high in P (summary of 13 trials; Satter and Wu, 1999).

	Low P		High P
Dietary P, % of diet DM	0.32–0.40		0.39–0.61
n	393		392
		Mean ± SD[a]	
Days to 1st oestrus	46.8 ± 10.9		51.6 ± 13.8
Days to 1st AI	71.7 ± 16.2		74.3 ± 10.6
Days open	103.5 ± 21.4		102.1 ± 13.0
Services per conception	2.2 ± 0.9		2.0 ± 0.5
Pregnancy rate	92% ± 6%		85% ± 5%

[a]Differences between means were not statistically significant for any measured variable.

inconsistencies between NRC requirements and the nutritional advice farmers receive. Undetected variation in the P content of feeds leads to imprecise ration formulation. Phosphorus content of forages analysed by the Northeast Diversity Health Institute Forage laboratory from May 1994 through April 1995 was highly variable (Kertz, 1998). The coefficient of variation in P content within forage type was 20 to 25%, and P content was more variable for grasses than for legumes. Despite this variation, wet chemistry analysis of forages for P content is not routinely requested.

Practical feeding recommendations influence P intakes in the field. Inconsistent recommendations from nutritionists, veterinarians and extension personnel have led many farmers to feed P in excess of the NRC recommendations. Dou *et al*. (2003) surveyed 612 dairy farms in New York, Pennsylvania, Delaware, Maryland and Virginia to assess dietary P concentration and to identify critical control points pertaining to P feeding management. Survey responses revealed a wide range of dietary P concentrations for lactating cows, from 0.36 to 0.70% of DM. The mean was 0.44% of DM, which was 34% above the concentration recommended by the NRC for 27.9 kg milk/day, the mean milk yield in the survey. Higher P concentrations in diets were not associated with higher milk yields, but increased dietary P led to higher P excretion in faeces. Most producers were feeding more P than cows needed because it was recommended in the rations by consultants. Until the environmental consequences became obvious, overfeeding P was viewed as cheap reproductive insurance. Revisiting the literature makes it clear that there is no documented benefit to overfeeding P.

One final reason P is overfed is the inclusion of feeds in the diet that are naturally high in P. Many by-product feeds are high in P, most notably the by-products of maize processing and ethanol production. These are increasingly popular feed supplements for beef and dairy cattle because of the protein and energy they supply. However, inclusion of these feeds in higher amounts often increases the dietary P content beyond the animal's requirement. Koelsch and Lesoing (1999) constructed nutrient balances for Nebraska livestock farms and found that producers who used these by-products had greater imbalances

between P inputs and outputs than producers who did not. In their study, the seven beef cattle operations that fed these products imported twice as much P into their farms as they exported in meat, crops and manure (input:output ratio of 2.0:1.0). In contrast, the nine farms who did not feed these products exported nearly as much P as they imported (input:output ratio of 1.1:1.0).

The popularity of these high P by-products will likely continue, and there is no easy solution to the problem of the resulting elevated dietary P, unless P is somehow removed before the by-product is sold to livestock farmers. In the short term, producers using these feeds should at least remove unneeded supplemental inorganic P from diets. In the long run, however, the true cost of the use of these high P feeds should be carefully considered. If the inclusion of these by-products will cause significant nutrient imbalance in the livestock operation and lead to difficulty meeting environmental regulations, then these feeds will not be as inexpensive as they appear as feed ingredients.

Crediting Bone Phosphorus Resorption in Early Lactation Cows

In lactating ruminants, opportunity exists to reduce P excretion by accounting for the P released through the normal catabolism of bone that occurs in early lactation. Resorbed bone is an available source of Ca and P that, if accounted for, would increase accuracy of dietary P recommendations and might reduce excretion of P. When sheep were fed diets varying in Ca and P contents throughout pregnancy and lactation (Braithwaite, 1983b), body P reserves were mobilized in late pregnancy and early lactation. This mobilization was in response to Ca requirements and was not affected by dietary P content.

Bone consists of Ca and P deposited within an organic collagen matrix. The highly porous nature of this matrix provides bone with an extensive surface area, making bone a highly labile source of both Ca and P. Bone mineral content is the result of the balance between rates of bone accretion and bone resorption. Bone accretion remains relatively constant within animals of a given age (Braithwaite, 1976, 1983a), so changes in bone mineral retention are due primarily to changes in rate of resorption.

Net bone resorption is likely a normal consequence in early lactation due to the rapid increase in demand for Ca to support milk yield. The postpartum ruminant is typically in negative Ca balance (Braithwaite, 1976), and parathyroid hormone is secreted in response to this hypocalcaemia (Shappell *et al*., 1987). Parathyroid hormone stimulates the conversion of 25-hydroxycholecalciferol to dihydrocholecalciferol. Together, parathyroid hormone and dihydrocholecalciferol stimulate bone resorption (Braithwaite, 1976; Ternouth, 1990).

Phosphorus release from bone during early lactation provides a readily available source of P to meet the needs for maintenance and milk yield. Ternouth (1990) suggested that beef steers fed P-deficient diets could mobilize up to 30% of bone mineral, or 6 g/day of P, meeting about half of their dietary requirement. Satter (2002) extended this estimate to a 600 kg lactating cow, and estimated that as much as 600 to 1000 g of P could be mobilized in early lactation. Supporting this, Knowlton and Herbein (2002) observed that apparent mobilization

of P from body reserves may meet a significant proportion of the dairy cow's net need for P during early lactation. Assuming that P balance reflects P resorption from bone, cows mobilized up to 25 g/day of P from bone in the first 3–5 weeks of lactation. The requirement for absorbed P in early lactation totals 45 to 70 g/day, depending on milk yield (NRC, 2001). Although Knowlton and Herbein (2002) reported no effect of diet on P retention, they observed an interaction between week of lactation and dietary P content, suggesting that dietary P affected the duration of net bone P resorption. Cows in all treatment groups mobilized P from body reserves at week 3 of lactation, but only cows fed the low P diet remained in negative P balance during week 5.

Taylor *et al*. (2009) evaluated Ca and P balance and mobilization from bone through 20 weeks of lactation to determine the timing and extent of net resorption of bone mineral and mineral balance in lactating dairy cows fed diets varying in dietary Ca. Dietary Ca concentration did not affect P balance through the 140-day study, but there was a clear effect of parity on balance, markers of bone metabolism and bone P concentration. Primiparous cows had higher serum osteocalcin (OC, a marker of bone formation) and higher serum deoxypyridinoline (DPD, a marker of bone resorption) than multiparous cows. Regardless of dietary Ca, serum OC concentration peaked around day 35 of lactation. Simultaneously, DPD concentration began to decrease, which may suggest a switch from net bone resorption to formation after day 35. However, this was not reflected in balance measurements. Reserves of P mobilized in early lactation must obviously be replenished in later lactation, and questions remain about the timing and rate of replenishment of bone P stores, as well as the duration and ultimate extent of mobilization of those reserves.

Availability of Feed Phosphorus

The availability (release and absorption) of P from feedstuffs affects the requirement for total dietary P in all species, but our assumptions of availability of feed P are based on relatively few studies. Questions remain for all species about appropriate methods and response variables to determine availability of feed P. Measurements of apparent P digestibility are not very useful as estimates of true (or net) availability of feed P because of considerable recycling of endogenous P via saliva.

Improved P availability from feed would allow the tissue-level needs of the animal to be met with reduced P intake. Dietary P absorbability is a function of release from the feed matrix and the degree to which released phosphate is absorbed at the small intestine. Because P intake and excretion are so tightly linked, the question of whether or not absorbability of feed P can be improved deserves attention.

Results from some studies suggest that the absorbability of phosphate from the small intestine is very high (greater than 90% in lactating goats fed below requirement; Koddebusch and Pfeffer, 1988). Efficiency of absorption of P from the small intestine increases as dietary P is reduced to or below the animal's true requirement, partly because salivary P becomes a greater and greater

proportion of total P entering the rumen-reticulum. Abundant flow of saliva contributes 70 to 80% of total endogenous P; the quantity recycled approaches that consumed from the diet daily (Horst, 1986). Salivary P recycling is an effective physiological conservation mechanism for ruminants and the potential absorbability of salivary P is proportionately greater than P from the diet (Challa *et al*., 1989).

Because absorption of phosphate from the small intestine is so high, any limitation to availability of feed P would be due to limitations on the release of P from the feed matrix. Approximately 65 to 70% of the total P in cereal grains is organically bound in phytate P (Nelson *et al*., 1968; Morse *et al*., 1992). This form of P is relatively unavailable to non-ruminant animals as they lack the enzyme phytase (Cromwell, 1992). Phytase catalyses the release of phosphate groups from the inositol ring of phytate, making the P more available for absorption in the small intestine (e.g. Cromwell *et al*., 1993; Lei *et al*., 1993; Kornegay and Qian, 1996; Jongbloed *et al*., 1997). Phytate P is more available to ruminants than to non-ruminants, because ruminal microorganisms possess phytase activity (Clark *et al*., 1986; Morse *et al*., 1992; Yanke *et al*., 1998).

The question of possible limitations of release of phytate P from the feed matrix is addressed in the literature via *in vitro* studies, *in vivo* experiments monitoring flow of phytate P to the small intestine or into the faeces and *in vivo* studies evaluating the effect of addition of exogenous phytase on digestion and excretion of P (or phytate P).

In vitro release of feed P from most feedstuffs is greater than 95% (e.g. Morse *et al*., 1992), but *in vivo* studies are more valuable because they reflect the impact of passage rate and P recycling. *In vivo* observations in sheep and goats indicate that grain type and ingredient processing affect ruminal P release and duodenal P flow. Bravo *et al*. (2003) reported that apparent ruminal P digestion in sheep ranged from 4.1 to 23.0%, depending on the ingredient. However, most estimates of apparent P digestion are much greater (NRC, 2001). Heat treatment of rapeseed meal increased the phytate in duodenal flow of sheep and decreased dietary phytate digestibility (Park *et al*., 2000). Similarly, ruminal P solubility (not the same, conceptually, as phytase activity or P release) of rapeseed meal and soybean meal decreased after formaldehyde treatment in lactating goats (Bravo *et al*., 2002) and in sheep (Park *et al*., 1999).

While endogenous activity of phytase in the rumen and reticulum is likely sufficient to fully release feed P from the feed matrix in normal healthy ruminants, physical properties of the diet and ruminal passage rates may prevent total hydrolysis of phytate in the rumen of lactating cows. Also, factors such as forage-to-concentrate ratio (Yanke *et al*., 1998; Knowlton *et al*., 2005), starch source and supplementation with purified phytic acid (Guyton *et al*., 2003) affect endogenous capacity to release organically bound P. Limited published research suggests modest reduction in faecal P excretion with addition of exogenous enzymes. Knowlton *et al*. (2007) added a blend of phytase and cellulase to the diet of lactating cows, and reduced faecal nutrient (N, P, DM) excretion was observed with similar milk yield. Similarly, Kincaid *et al*. (2005) reported that hydrolysis of phytate P was increased by exogenous phytase, and total P digestibility tended to be increased. Bravo *et al*. (2003) reported that fungal phytase

addition increased P digestibility of formaldehyde-treated soybean meal and formaldehyde-treated sunflower meal.

Concern about or consideration of sufficient native microbial phytase activity in the rumen and reticulum for release of feed phytate P might be especially relevant any time a major shift in dietary ingredients occurs and the microbial population is undergoing change. Such might be the case when non-lactating, pregnant cows are transitioned from a prepartum diet to one for lactation. Native or supplemental phytase activity in this situation has not been studied. At such times, efficient reabsorption of recycled salivary P is doubtless especially important. Overall, if total dietary P can be fed as close to the true requirement as possible a larger proportion of P absorbed will be of salivary origin and the efficiency of absorption will be improved, reducing P excretion. This will benefit environmental management of P. It seems unlikely, given the tremendous benefit of salivary recycling of P as an evolved conservation mechanism, that ruminants are ever at much nutritional risk of P insufficiency through the typical production cycle.

Implications

Phosphorus-based nutrient management regulations increase the amount of land required to dispose of manure, and that will have a detrimental effect on the economy in areas of intensive animal agriculture. Opportunities are now available to reduce P excretion by ruminants by 25 to 40% by more accurate feed analysis and ration formulation, and more precise mixing and feeding of rations to meet the animals' actual dietary P requirements. Reducing the P content of manure through nutrition is a powerful, cost-effective approach to reducing P losses from livestock farms.

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9 Dynamics of Calcium and Phosphorus Metabolism in Laying Hens

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Introduction

In poultry, calcium (Ca) and phosphorus (P) are involved in several biological processes, such as bone development and eggshell formation. Broilers, selected for rapid growth, require high levels of Ca and P for skeletal development and energy metabolism during early life. Calcium and P deficiencies in these fast-growing lines can lead to skeletal abnormalities such as tibial dyschondroplasia (TD), which is a common cause of deformity, lameness and mortality in broilers (e.g. Edwards and Veltmann, 1983; Edwards, 2000; Fleming, 2008).

In layers, Ca and P availability is most crucial during the laying period. Egg-laying hens need large amounts of Ca to deposit in the egg and, mainly, its shell. The larger part of eggshell formation takes place during the dark hours (scotophase), when hens have their nocturnal fast (Scanes *et al*., 1987) and Ca supply from the digestive system is low. At these moments the hen relies on an ingenious system that allows her to mobilize previously accumulated Ca stores from medullary bone tissue (Mueller *et al*., 1964; Etches, 1987). Since Ca is stored almost entirely as hydroxyapatite crystals of Ca phosphate ($3Ca_3(PO_4)\cdot Ca(OH)_2$), mobilization of Ca results in concurrent release of P (Whitehead and Fleming, 2000). This can lead to elevated plasma levels of P, which will be excreted in the urine (Hurwitz and Bar, 1965). Thus, excretion of P with poultry manure into the environment does not just depend on P intake levels and P output in eggs, but also on dietary Ca levels. Excess P in poultry manure may contribute to build-up of P in the soil and eutrophication (Sharpley, 1999). If either dietary Ca or P is limiting, proper utilization and storage of these minerals are impossible. In the reverse situation, when there is an excess of one of both minerals, the availability of the other may be reduced due to the formation of insoluble Ca phosphate in the intestine (Underwood, 1981). Deficiencies in Ca, P or both may result in reduced bone and eggshell quality (Clunies *et al*., 1992;

 Phosphorus and Calcium Utilization and Requirements in Farm Animals (D.M.S.S. Vitti and E. Kebreab)

Fleming, 2008). In addition, excess levels of Ca and P may interfere with other nutrients, e.g. zinc and magnesium, thereby causing a deficiency of these elements (Maynard *et al.*, 1979).

The first part of this chapter focuses on the homeostatically regulated Ca and P metabolism. In the latter part, a dynamic model of Ca and P dynamics in layers is presented that could help in evaluating the effects of changes in feeding management of laying hens.

Calcium, Phosphorus and Vitamin D_3

Calcium

Calcium is ingested with the feed and absorbed primarily in the duodenum and upper jejunum into the vascular system (Hurwitz and Bar, 1965). The extracellular Ca pool continually contains 90–120 mg Ca/l, from which Ca needed for metabolic processes, bone development, growth and productivity is constantly released (Maynard *et al.*, 1979; Underwood, 1981). In egg-laying hens, plasma Ca is increased to 200–300 mg/l (Maynard *et al.*, 1979; Underwood, 1981; Etches, 1987) to facilitate the high Ca flow to the shell gland.

Dietary Ca can be obtained from inorganic or organic sources. The bioavailability of Ca from these different sources has been extensively discussed (reviewed by Shafey, 1993). In the diet, it can be present in a fine (e.g. limestone) or coarse (e.g. oyster shell) form. There are indications that feeding coarse Ca sources may have a positive effect on feed intake, body weight, bone mineralization and eggshell quality (reviewed by Saunders-Blades *et al.*, 2009). These authors, however, were not able to detect the same effects on eggshell quality as found in other studies. This might be due to the relative low large particle contents of the diets used in their study. Nevertheless, it is clear that particle size might play an important role in adequate Ca uptake.

Phosphorus

Phosphorus is needed mostly for skeletal development, but it also plays a considerable role in energy metabolism. Phosphorus absorption takes place primarily at the duodenum and jejunum (Hurwitz and Bar, 1965). Phosphorus levels in the extracellular P pool usually range from 4 to 9 mg/dl (Maynard *et al.*, 1979). Dietary P can be obtained from plant and animal feedstuffs and from inorganic supplements. In plant and animal feedstuffs P is present as either organically bound salts of phytic acid (phytate P) or other forms (non-phytate P). Monogastrics have more difficulties digesting phytate P compared with non-phytate P, especially when Ca content in the feed is high (Simons, 1986), although both broilers (Van der Klis and Versteegh, 1995) and layers (Van der Klis and Versteegh, 1996) are capable of breaking down a fraction of it. The bioavailability of different inorganic phosphate sources has been investigated in many studies (reviewed by Waldroup, 1999) and is also covered in Chapter 6. Mono- and dicalcium

phosphates have the highest biological value and are most commonly used in diet formulation.

Vitamin D

Vitamin D_3 (cholecalciferol) is essential for absorption of Ca and P. It can be obtained either directly from the diet or it can be synthesized from its precursor, 7-dehydroxycholesterol, which is formed in the liver. 7-Dehydroxycholesterol is then transported to the skin, where it is transformed to vitamin D_3 under the influence of ultraviolet light and skin temperature.

In the liver, and to a lesser extent in the kidney and intestines, vitamin D_3 is transformed to 25-hydroxyvitamin D_3 (25-OHD_3). In the kidney, and to a lesser extent in other tissues, including intestine, bone and skin, this is converted into 1,25-$(OH)_2D_3$, which is the hormonal form of the vitamin. The conversion takes place with the help of 1-α-hydroxylase and is homeostatically regulated by plasma Ca^{2+}, the secretion of parathyroid hormone (PTH) and possibly also by plasma P, calcitonin and the secretion of gonad hormones (e.g. Soares, 1984). Recently, vitamin D metabolism has been reviewed extensively by Bar (2008).

Regulation of Calcium and Phosphorus Metabolism

In order to ensure a proper distribution of available Ca and P over the required processes, while keeping Ca and P in the extracellular pool at a constant level, both laying and non-laying birds exhibit a complex mechanism. This mechanism helps the bird to maintain homeostasis, even under suboptimal nutrient conditions. In the case of persistent deficiencies, however, homeostasis is disturbed and one or more processes will be confined.

Calcium and P homeostasis is maintained through a complex feedback system, described and discussed by several authors (e.g. Soares, 1984; Etches, 1987; Rao and Roland, 1990; Newman and Leeson, 1997; DeLuca, 2004; Bar, 2008, 2009), and which is illustrated in Fig. 9.1. When plasma Ca^{2+} declines, due to drain of Ca by different biological processes, the parathyroid gland is stimulated to secrete PTH. Parathyroid hormone, but also 17-β-oestradiol, prolactin and growth hormone (GH), stimulate the hydroxylation of 25-OHD_3 to 1,25-$(OH)_2D_3$ in the kidney. This hydroxylation is also directly stimulated by low plasma Ca^{2+}. 1,25-$(OH)_2D_3$ and PTH alter Ca and P metabolism via three pathways:

1. Intestine. 1,25-$(OH)_2D_3$ stimulates the efficiency of absorption of Ca and P in the intestine.
2. Bone. 1,25-$(OH)_2D_3$, together with PTH or GH, stimulates the degradation of medullary bone, so that Ca and P can be released into the blood. In the absence of both PTH and GH, when plasma Ca^{2+} and P levels are at required levels, 1,25-$(OH)_2D_3$ stimulates bone mineralization.
3. Kidney. 1,25-$(OH)_2D_3$, together with PTH, has a stimulating effect on the resorption of Ca and a suppressing effect on the resorption of P in the kidney.

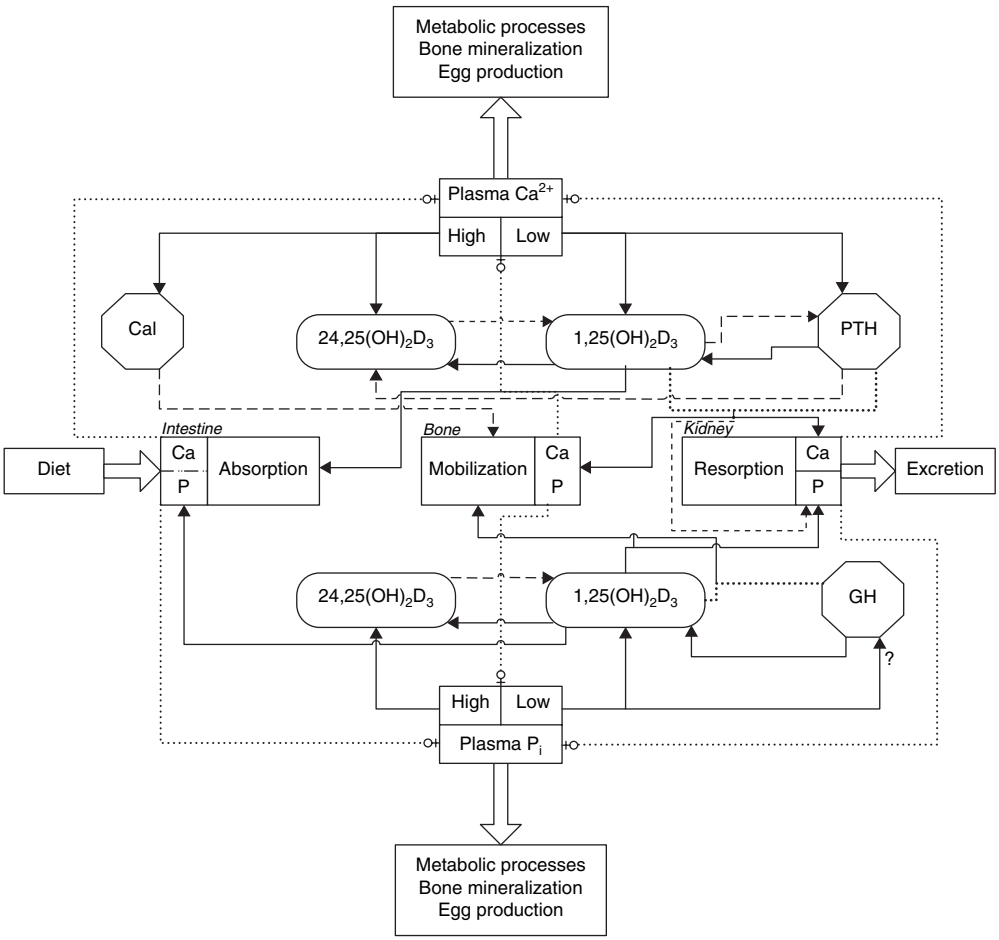

Fig. 9.1. Calcium and P homeostasis in the laying hen. Adapted and modified from Rao and Roland (1990) and Bar (2008). Open arrows (⇧) indicate Ca and P demand and supply processes. Solid lines (–) indicate positive regulation, whereas dashed lines (---) indicate that negative regulation takes place. Dotted lines (**...**) indicate co-regulation of different hormones. Light dotted lines (...) show the effect of Ca and P absorption, resorption and mobilization on plasma Ca^{2+} and P levels. Cal, calcitonin; GH, growth hormone; PTH, parathyroid hormone. Note that 17-ß-oestradiol and prolactin are also involved in the regulation of $1,25\text{-}(OH)_2D_3$ and $24,25\text{-}(OH)_2D_3$. See text for detailed discussion.

Consequently, it reduces Ca excretion and increases P excretion in the urine. The effect of $1,25\text{-}(OH)_2D_3$ on renal resorption in the absence of PTH is not completely clear yet and will be discussed later.

In addition, $1,25\text{-}(OH)_2D_3$ also stimulates the production of other enzymes, binding proteins and membrane components that are involved in the transport of Ca and P (Frost *et al.*, 1990). Along with indirect stimulation via $1,25\text{-}(OH)_2D_3$, the last two processes also require direct stimulation by PTH. In birds, direct

stimulation by PTH acts rapidly, whereas regulation via 1,25-$(OH)_2D_3$ appears more slowly. There has been some controversy about the effect of 1,25-$(OH)_2D_3$ on the parathyroid gland (Johnston and Ivey, 2006). It is, however, most likely that 1,25-$(OH)_2D_3$ suppresses PTH secretion (Jones *et al.*, 1998; DeLuca, 2004). Elevated plasma Ca^{2+} levels result in release of calcitonin from ultimobranchial glands. Calcitonin blocks the mobilization of Ca and P from the skeleton, thereby suppressing plasma Ca^{2+}. Although some involvement of calcitonin in the regulation of vitamin D_3 metabolism has been described, it is not clear if calcitonin plays a direct role (reviewed by Jones *et al.*, 1998; Johnston and Ivey, 2006).

Also, when plasma P declines, levels of 1,25-$(OH)_2D_3$ in the plasma and target tissues increase, independently of PTH (Tanaka and DeLuca, 1973; reviewed by Newman and Leeson, 1997; Bar, 2008). It is unclear, however, how regulation of 1,25-$(OH)_2D_3$ by plasma P levels takes place. As reviewed by Bar (2008), low levels of dietary P do not seem to induce renal 1-hydroxylation of 25-$(OH)D_3$. According to Bar (2008), this could be a result of retarded clearance of 1,25-$(OH)_2D_3$ or an increased affinity of the vitamin D receptor. A third possibility might be that dietary P restriction induces 1-hydroxylation of 25-$(OH)D_3$ not in the kidney but in another tissue, e.g. intestine, bone or skin. Unlike low plasma Ca^{2+}, low plasma P does not induce the secretion of PTH, and hence regulation of mobilization and resorption does not occur via the same pathways. While it is known that 1,25-$(OH)_2D_3$ stimulates degradation of medullary bone only in the presence of PTH or GH, it is most likely that hypophosphataemia induces GH release, in order to increase plasma P. There are indications that, in the absence of PTH, 1,25-$(OH)_2D_3$ might stimulate renal P resorption. However, it is still not clear how this regulation takes place exactly and which factors are involved. While this mechanism only involves the slowly acting 1,25-$(OH)_2D_3$, and not the more rapidly acting PTH, it might be expected that the plasma P initiated mechanism has a longer response time compared with the plasma Ca^{2+} mechanism.

Both high plasma Ca^{2+} and P seem to induce the production of 24,25-$(OH)_2D_3$ from 25-$(OH)D_3$ but also from 1,25-$(OH)_2D_3$ (24-hydroxylation). Parathyroid hormone and 17-ß-oestradiol suppress 24-hydroxylation, whereas 1,25-$(OH)_2D_3$ levels stimulate the process. The role of 24,25-$(OH)_2D_3$ is not completely understood, but it is clear that formation of this metabolite affects plasma 1,25-$(OH)_2D_3$ and thus might be involved in vitamin D_3 regulation and inactivation (Jones *et al.*, 1998).

How the withdrawal of Ca from the blood to the uterus for eggshell formation is initiated and which factors are involved are still unclear. It is suggested that gonad hormones, secreted in a circadian rhythm coupled with the egg cycle, are involved. An undefined endocrine factor could be responsible for the onset of Ca deposition in the eggshell, whereas it is most likely that progesterone regulates the offset (Bar, 2009).

Calcium and Phosphorus Metabolism During Egg Formation

Approximately 99% of all Ca in the egg can be found in the shell, whereas most P is present in the yolk. Yolk and albumen formation are assumed to be

constant during the day, whereas eggshell formation starts approximately 4 hours after ovulation. Phosphorus requirements for egg production can therefore be considered to be constant over the day, similar to constant P requirements for growth and maintenance. Calcium requirements, however, vary considerably over the day due to the dynamic process of eggshell formation during the day.

The larger part of eggshell formation takes place during the scotophase, especially when ovulation occurs later in the day (for detailed calculations, see Van Krieken, 1996). Dietary Ca supply is low during these dark hours, particularly later in the night when there is almost no feed left in the gut. As a result, Ca has to be mobilized from the medullary bone reserves in order to fulfil the high requirements. Consequently, a considerable proportion of Ca in the eggshell is derived from bone reserves (up to 20–40%, as reported by Bar, 2009). Degradation of the Ca-bearing hydroxyapatite from the bone leads to elevated plasma P levels due to concurrent release. These excess levels of P cannot be utilized at that moment and will be excreted with the urine.

At the commencement of the photophase, feed intake is started again and plasma Ca^{2+} and P levels increase. At the same time, eggshell formation comes to an end and less Ca is needed. The remaining Ca can now be deposited in the bone, provided that there is enough P available to be stored together. The moment of providing Ca and P as well as the ratio with which they are provided determine, therefore, whether these nutrients will be utilized directly, deposited in the bone or excreted.

Hence, if given a choice of feeds, it is not remarkable that the hen reduces her Ca intake during the morning when Ca requirements are low, and shows a preferential Ca uptake at the end of the photophase (Chah and Moran, 1985; reviewed by Etches, 1987) on days when shell formation takes place (Mongin and Saveur, 1974; reviewed by Sykes, 1984). Feeding an identifiable free-choice Ca^{2+} source may give the hen the opportunity to adequately reduce or increase her Ca intake at essential moments. Voluntary P intake is, in contrast, higher in the morning compared with the intake in the afternoon (Keshavarz, 1998). This is probably due to the increased necessity to store P, together with Ca, in medullary bone.

In addition to these circadian changes in Ca requirements for egg production, Ca requirements are also affected by the stage of ovulatory sequence. The ovulatory sequence of a hen usually consists of three to nine ovulatory cycles and is followed by a rest day on which no oviposition occurs. Each cycle takes approximately 24–28 h, starting with ovulation and ending with oviposition. Each oviposition, with the exception of the last one, is followed by an ovulation of the next cycle within 1 h. The first oviposition in sequence occurs within 3 h from the start of the photophase. Each successive oviposition occurs slightly later in the day. A rest day follows when oviposition occurs some 7 h from start of the photophase period (Etches, 1987). On non-ovulation days, when there is no eggshell formation, and the day after, feed intake and Ca absorption are reduced in order to reduce Ca uptake (Hurwitz, 1973; Etches, 1987; Scanes *et al.*, 1987; Bar, 2009).

Calcium and Phosphorus Supply

From Fig. 9.1 it is clear that blood plasma levels of Ca^{2+} and P are restored either from exogenous Ca and P absorbed from ingested feed or, when this is not available, from endogenous sources mobilized from medullary bone. The quantity of Ca and P available from exogenous sources depends on feed intake, passage rate of the chyme through the gastrointestinal tract, the presence of other dietary components in the gastrointestinal tract and the absorption capacity of the different intestinal segments. Mobilization of bone reserves occurs when plasma Ca^{2+} or P cannot be maintained above certain threshold levels and is regulated via the production of 1,25-$(OH)_2D_3$ from 25-$(OH)D_3$ as described in the previous section.

Absorption of calcium and phosphorus in the intestine

The efficiency with which Ca and P are absorbed from the intestine depends on the quantity and form of these elements in the feed. Low Ca and P intake will result in relatively low plasma levels of these elements, and absorption in the intestine will be increased (Hurwitz and Bar, 1969; Clunies *et al.*, 1992). In egg-laying hens the absorption of Ca and P also depends on the stage of eggshell formation. The studies of Hurwitz and Bar (1965, 1969) illustrate that, when Ca intake meets minimal requirements, 68 and 72% of available Ca in the chyme is absorbed during early and late stages of eggshell formation, whereas this is only 40% when there is no eggshell formation. In the case of low Ca intake, relative absorption can be increased to levels over 80% in periods of eggshell formation, but remains at 40% when there is no eggshell formation (Bar, 2009).

Phosphorus absorption increases as well during periods of eggshell formation, although not as strikingly as Ca absorption (Hurwitz and Bar, 1965). This seems legitimate since the quantity of P needed in the eggshell is less than 0.1% of the total P requirements (calculated based on values of the National Research Council, 1994). A factor that does have a considerable effect on the efficiency of P absorption is the quantity of Ca in the feed (Hurwitz and Bar, 1965). High levels of dietary Ca result in high plasma Ca^{2+}, which results in reduced P absorption, whereas low plasma Ca^{2+} results in increased absorption.

Renal 1-hydroxylation decreases with age, resulting in lower 1,25-$(OH)_2D_3$ levels in older animals. As a consequence, Ca absorption in the intestine decreases, which may be responsible for the reduction in bone and eggshell quality observed in older hens (Al-Batshan *et al*., 1994). After induced moult, Ca absorption and thereby bone and eggshell quality could be increased again, possibly due to increased renal 1-hydroxylase and thus increased levels of 1,25-$(OH)_2D_3$.

Mobilization of calcium and phosphorus from medullary bone

The skeleton of laying hens becomes fully developed during the rearing period. The main bone types contributing to this structure are cortical and cancellous bone, both forms of lamellar bone. After formation, bones are maintained by a

continuous process of bone deposition by osteoblasts and bone resorption by osteoclasts. An imbalance between these processes can result in net loss of structural bone, which can lead to metabolic disorders, such as osteoporosis (Whitehead and Fleming, 2000). At onset of sexual maturity, increased levels of oestrogen trigger the osteoblasts to completely switch to formation of soft, woven bone tissue, called medullary bone (Dacke *et al.*, 1993; Whitehead, 2004) and the formation of structural bone ceases (Hudson *et al.*, 1993). Osteoclastic activity is slowed down by oestrogen but is increased again during periods of shell formation. Resorption of structural bone therefore continues during the laying period and, as a result, the quantity of structural bone gradually decreases. In addition, there are indications that, in the case of long term Ca deficiency, medullary bone reserves are restored from structural bone (Dacke *et al.*, 1993). This gradual bone loss in layer hens is associated with osteopenia and osteoporosis (Whitehead, 2004; Fleming, 2008). Optimizing structural bone content of the hen before onset of maturity might help to buffer the effects of the loss of this structural bone during lay. This emphasizes the importance of adequate nutrition, especially the inclusion of appropriate levels of Ca, P and vitamin D during the rearing period (Whitehead and Fleming, 2000; Fleming, 2008).

Medullary bone serves as a temporary Ca depot, from which Ca can be released during shell formation, when requirements are high and exogenous supply is low (Mueller *et al.*, 1964; Etches, 1987). Its formation occurs rapidly during the early laying period and continues slowly during the remainder of the laying period (Whitehead, 2004).

Calcium and Phosphorus for Optimal Bone and Eggshell Quality

In the previous section, Ca and P metabolism and its ways of regulation, as well as its role in the different processes in the body, have been described. In current laying hen practice, bone and eggshell quality, as well as P excretion, is of considerable importance and adequate measures to optimize these aspects are needed. In the last few decades, various empirical studies have pointed out methods to optimize one or more of these aspects (e.g. Rao and Roland, 1990; Keshavarz, 1998; Rama Rao *et al.*, 2006; Nahm, 2008; Saunders-Blades *et al.*, 2009). These methods include management strategies to match Ca and P intake with the requirements during the day, and diet formulation to optimize Ca and P content and availability.

As an example, Table 9.1 summarizes the results of four studies in which the effects of different dietary Ca and P levels and ratios on bone and eggshell quality were investigated. The results of these and other studies show that both Ca and P content of the diet, as well as the ratio in which these minerals are present in the diet, can have considerable effects on bone and eggshell quality of layers. But they also reveal that the exact requirements for both Ca and P are not completely clear yet, due to interfering factors such as oviposition time, solubility of the Ca source, light schedule, differences in feed intake pattern and gut physical and metabolic conditions, which influence Ca and P availability throughout the day (Van Krieken, 1996; Tolboom and Kwakkel, 1998).

Table 9.1. Shell specific gravity, shell thickness, bone ash and bone mineral content of layer hens fed different levels and ratios of Ca and P.

Ca/avP (totP)[a] content of the feed (%)	Ca:P ratio	Shell specific gravity	Shell thickness (mm)	Bone ash (%)	Bone mineral content (g/cm)	Reference
2.75/0.30	9.2	1.0843		43.8	0.190	Frost and Roland (1991)
2.75/0.40	6.9	1.0841		46.5	0.205	21–31 weeks
2.75/0.50	5.5	1.0830		45.4	0.211	
3.75/0.30	12.5	1.0869		47.1	0.220	
3.75/0.40	9.4	1.0862		46.1	0.219	
3.75/0.50	7.5	1.0863		46.1	0.225	
4.25/0.30	14.2	1.0875		46.7	0.227	
4.25/0.40	10.6	1.0871		46.6	0.229	
4.25/0.50	8.5	1.0868		46.5	0.231	
2.00/0.28(0.50)	7.1	1.074				Keshavarz and Austic (1990)
2.00/0.78(1.00)	2.6	1.074				38 weeks
3.50/0.28(0.50)	12.5	1.082				
3.50/0.78(1.00)	4.5	1.080				
4.00/(0.33)	12.1	1.0884			0.143	Gordon and Roland (1997)
4.00/(0.43)	9.3	1.0888			0.157	21–38 weeks
4.00/(0.53)	7.6	1.0885			0.159	
4.00/(0.63)	6.3	1.0880			0.159	
4.00/(0.73)	5.5	1.0878			0.146	
3.25/0.28	11.6		0.385	51.9		Rama Rao *et al.* (2003)
3.50/0.28	12.5		0.379	53.2		28–48 weeks
3.75/0.28	13.4		0.386	53.5		
4.00/0.28	14.3		0.391	53.5		
4.25/0.28	15.2		0.386	53.6		
4.50/0.28	16.1		0.389	53.3		

[a]avP = available P, totP = total P.

Dynamic Modelling of Calcium and Phosphorus Metabolism in Layers

From the previous sections, it is clear that the supply and requirements of Ca and P are strongly interrelated. An imbalance in availability of Ca may result in excess amounts of P to be excreted and vice versa, and especially the surplus of P in poultry manure presents an environmental problem for intensive poultry

operations. Nutritional requirements of the hen for P vary from 2.0 to 3.5 g of non-phytate or available P/kg of diet during peak lay (Boorman and Gunaratne, 2001). Such uncertainty regarding P requirements is related to, among other things, the close relationship between P and Ca dynamics in layers and the wide range in oviposition times, and hence in P and Ca requirements of the layer within the day. Mathematical models of digestion and metabolism in farm animals have been widely used to understand these processes quantitatively and predict excretion of nutrients and minerals in manure (Dumas *et al*., 2008). To describe mineral flows in layers quantitatively, a number of models have been developed. Etches (1987) predicted Ca dynamics on an hourly basis but did not consider P in the model. Van Krieken (1996) and Tolboom and Kwakkel (1998) modelled Ca and P dynamics in layers, partly based on the Etches (1987) model, also on an hourly basis, in which most transactions were linear. However, in biology most fluxes are enzyme related and non-linear, and in describing the nutrient dynamics of the system and its underlying components, differential equations are often used. The mathematically standard way of representing such models is the rate:state formalism (Thornley and France, 2007). A new dynamic, mechanistic model of P and Ca metabolism in layers was developed by Kebreab *et al*. (2009) to simulate diurnal changes for Ca and P and the hourly requirement of the layer for those minerals, with special focus on the excretion of absorbed P in manure. This model is briefly described below and results of various management options for Ca and P dynamics presented.

Model description

The model is fully described by Kebreab *et al*. (2009). The model consists of eight state variables representing Ca and P pools in the crop, stomachs, plasma and bone. In line with model objectives, P is defined as absorbable P at the terminal ileum. Zero pools are assigned to Ca and P in the duodenum, assuming that the retention time of Ca and P in the duodenum is very small. Differential equations describe the rate of change of the state variables in time. The model was programmed in SMART simulation software (Kramer and Scholten, 2001). Euler's method of integration was used with a step size of 0.6 minutes and was run for 5 days to ensure that a quasi-steady state was achieved.

In the model, oviposition occurs at 1, 2, . . . or 7 h after the light is switched on, or the hen may have a pause day the day after oviposition at 7 h. The standard light period is set at 16 h/day. Feed intake was assumed to be 101% of average intake on all days, except for the rest day and the day immediately before the rest day, where intake was 90% of average intake, as described in the previous sections. In the default situation, feed intake is 110 g/day with Ca and absorbable P levels of 40 and 2.8 g/kg feed, respectively. The live weight of the hen is 1.7 kg, egg weight is 59 g and rate of lay is 95 eggs per 100 days. These values can be changed by the user.

Feed intake was assumed to occur only during the period when the light is on and assumed to be continuous during this photoperiod. Outflow from the crop to the stomachs and from the stomachs to the duodenum is according to

mass-action kinetics. As described in the previous sections, absorption of Ca from the duodenum during eggshell formation is higher than when there is no eggshell formation, and Ca absorption in these periods is assumed to be 70 and 40%, respectively, of inflow into the duodenum. There are two inputs each to the plasma Ca and P pool, namely absorbed Ca and P from the duodenum and mobilized Ca and P from bones. Three outputs each from the plasma Ca and P pools are represented, namely Ca and P utilization for egg synthesis (sum of eggshell, egg white and yolk), Ca and P utilization for deposition in bones and Ca and P excretion with urine. Eggshell formation is assumed to occur in the 20 h prior to oviposition (Etches, 1987) in a sigmoidal pattern, where rate of synthesis is represented by the derivative of a logistic function (Fig. 9.2) (Van Krieken, 1996). For bone synthesis and mobilization, a fixed Ca:P ratio of 2.2:1 is assumed, since Ca and P in bones are stored as hydroxyapatite crystals of Ca-phosphate. The amount of Ca and P used for bone synthesis is represented as a saturable (Michaelis–Menten kinetics) process that depends on plasma Ca or P concentration, in which actual deposition is taken as the lowest synthesis rate calculated from Ca or from P availability. Similarly, bone mobilization is represented as a saturable process based on availability of Ca and P in the plasma, and taken as the highest mobilization rate calculated from plasma Ca and P availability. Thus, this representation ensures that bone synthesis occurs at a rate determined by the most limiting of the two minerals, and similarly bone mobilization occurs at a rate determined by the highest demand for one of the two minerals. Finally, Ca and P excreted in the urine are the sum of: (i) the maintenance requirement for Ca and P; (ii) the amount of Ca or P in the plasma that cannot be used for bone synthesis because the other mineral (P and Ca, respectively) is lacking; and (iii) the amount of Ca or P released from bone because P or Ca is required for maintenance and egg synthesis. Hence any mineral not used for bone synthesis

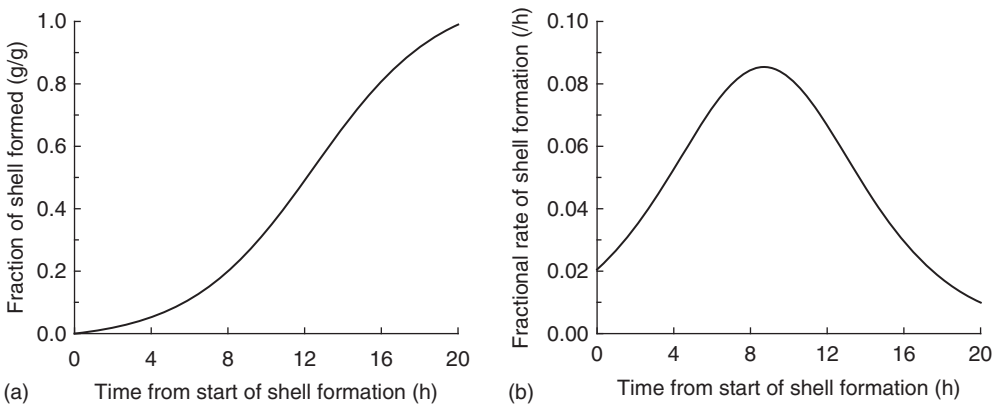

Fig. 9.2. Time course of eggshell formation. (a) Cumulative eggshell formation (fraction of total). (b) Fractional rate of eggshell formation (derivative of cumulative eggshell formation) (/h). Eggshell formation (fraction of total) is $1.11/(1 + e^{-0.3077\ (\text{time} - 8.5)}) - 0.08$; parameters estimated by Van Krieken (1996) based on data collected from literature and reported by Etches (1987).

because availability of the other mineral is not sufficient to support that synthesis is excreted in urine. Equally, any mineral that has been mobilized because of necessary mobilization of the other mineral is excreted in urine.

The model endeavours to integrate existing concepts and data in a format compatible with dynamic, quantitative analyses. As with any model, assumptions have to be made and model parameters derived. All of these are open to scientific debate. The model helps to indicate areas where current knowledge is inadequate, and evaluating the effects of changes in parameter values (sensitivity analyses) is useful to understand the effects of such alterations in the system under study.

Model application

Diurnal changes in Ca and P for oviposition at 1 h, 4 h or 7 h after the light is switched on and for a pause day are presented in Fig. 9.3. From the moment the light is switched on (at 0 h), feed intake commences and, consequently, Ca and P absorption increases. After 16 h, the light is switched off and feed intake ceases and Ca and P absorption declines, because the amounts of Ca and P present in crop and stomach quickly decrease. If oviposition is at 1 h after the light is switched on (Fig. 9.3a and b), Ca requirements are small from Hour 1 until Hour 5, when formation of a new eggshell starts. Since the majority of P is required for synthesis of egg yolk, assumed to be a continuous process, P requirements are more equal during the day. In the first hour, P requirements are higher than P absorption, and therefore P and Ca mobilization from bone occurs. In this hour, P excretion in urine is merely related to maintenance requirements. In contrast, mobilized Ca cannot be utilized or stored and is therefore excreted in urine. After eggshell formation starts, Ca requirements increase and decrease in a pattern related to that of eggshell formation (compare Ca requirements in Fig. 9.3a with eggshell formation in Fig. 9.2b). Simulated Ca requirements are highest at about 11–17 h after previous oviposition, whereas Ca supply decreases after Hour 16. Calcium absorption is sufficient to meet Ca requirements until Hour 18. Phosphorus absorption is sufficient to meet requirements until Hour 20. However, the surplus of P absorbed cannot always be utilized for bone synthesis, since Ca may be lacking to support this synthesis. That is the case during approximately Hours 11–18. Hence, a part of absorbed P is not utilized in these hours and is excreted in urine. From Hour 18 until the end of the dark period, Ca has to be mobilized to support requirements, giving rise to P mobilization as well. A large part of this mobilized P is not required for maintenance or egg synthesis and is consequently excreted in urine. Thus there are two reasons why P is unutilized and excreted in the urine. During Hours 11–18, P uptake in feed and therefore P absorption from the gut is too high relative to Ca availability to support high bone synthesis rates. During Hours 18–24, P that is not utilized largely originates from bone mobilization because of Ca requirements.

The simulations indicate that, when oviposition is at 1 h after the light is switched on, only about 44% of dietary available P is utilized for maintenance or deposited in the egg, and 22% is deposited in bone. Therefore, approximately

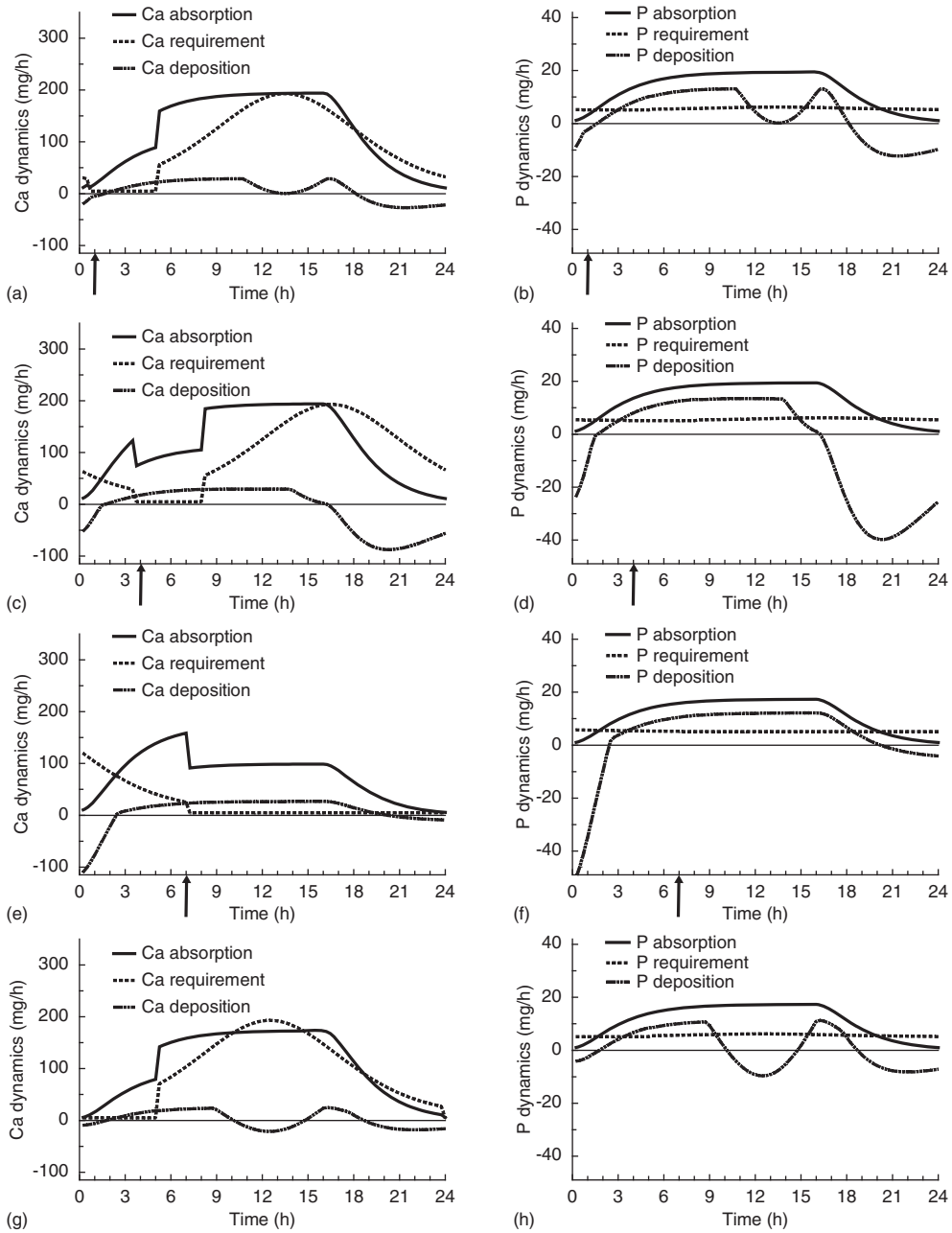

Fig. 9.3. Simulated diurnal dynamics of Ca and P in a layer at oviposition 1 h (a, b), 4 h (c, d), 7 h (e, f) after light is switched on or at a pause day (g, h). An arrow denotes time of oviposition. Ca or P absorption is absorption from the duodenum, Ca or P requirement is requirement for maintenance and egg production, and Ca or P deposition is bone synthesis (positive values) or bone mobilization (negative values).

one-third of available P intake is not utilized (107.9 mg/day; Table 9.2) because of instantaneous deficits of Ca. This would also indicate that dietary absorbable P can be reduced by 22% to obtain a zero bone P balance and reduce P excretion into the environment. However, evaluation of feeding strategies requires simulations at all possible hours of oviposition and assumptions on the frequency distribution of those hours within a flock of layers, described later.

When oviposition occurs at later times after the light is switched on, relatively more of the shell-forming process occurs during the night. Hence, Ca requirements occur especially during those hours when Ca supply from the gut is small, as illustrated for oviposition 4 h after the light is switched on in Fig. 9.3c and d. Simulated Ca requirement is much higher than Ca absorption from Hour 16 onwards due to a high shell synthesis rate and a declining rate of Ca absorption, giving rise to bone mobilization. Consequently, large amounts of P are mobilized as well and excreted in urine. Mobilization of bone in these dark hours is not compensated by synthesis of bone during the light hours, and there is net bone mobilization on this day of 102.4 mg P/day (see Table 9.2). In this case, dietary absorbable P cannot be reduced because of bone depletion that has already occurred. Ca and P dynamics of a hen for oviposition at Hour 7 and for a rest day are shown in Fig. 9.3e to h. Even though feed intake is lower on the day when oviposition occurs at Hour 7, the hen is in positive Ca and P balance during most of the day as no new egg will be formed that day. Therefore, P losses due to imbalance of Ca and P are small and bone synthesis (P deposition in bone of 89.1 mg/day) helps to provide Ca and P storage for the next series of eggs. On a rest day, there is no oviposition but Ca demands are high to form the eggshell for the next day. Given the lower Ca intake level on a rest day, there is bone mobilization during peak shell synthesis rates as well as during most of the dark hours. The amount of P not utilized is therefore higher than on a day with oviposition at Hour 1 (123.5 and 107.9 mg/day, respectively).

Table 9.2. Simulated averaged P flow in a 100-day period and simulated daily P flows (all in mg/day) in layers at various oviposition times after the light is switched on. The total excretion of P in urine can be calculated as the sum of P for maintenance and P not utilized.

Oviposition (h)	Days/ 100 days	Absorbed	Maintenance	Into egg	Into bone	Not utilized
1	15	311.1	23.8	111.6	67.8	107.9
2	16	311.1	23.8	111.6	8.4	167.3
3	20	311.1	23.8	111.6	–48.5	224.2
4	17	311.1	23.8	111.6	–102.4	278.1
5	12	311.1	23.8	111.6	–152.5	328.2
6	10	311.1	23.8	111.6	–198.4	374.1
7	5	277.2	23.8	101.4	89.1	62.9
Pause day	5	277.2	23.8	111.3	18.6	123.5
Weighted average		307.7	23.8	111.1	–48.3	221.1

To calculate total Ca and P flows during a longer period, assumptions about the frequency distribution of hours of oviposition within a flock of layers have to be made. Average P flows in a layer are presented in Table 9.2 assuming a frequency of laying times as reported by Van Krieken (1996). Such a frequency distribution of laying times may easily be adapted to analyse different frequency distributions and rates of lay. The simulations indicate that on average more than two-thirds of absorbable P intake (221.2 mg P/day) is not utilized because of Ca shortage and is excreted with urine. Mobilization of P from bone is on average 48.3 mg/day, which corresponds to mobilization of Ca of 106.3 mg/day. Given such a level of bone mobilization, osteoporosis problems may occur, as described in the previous section.

The model provides a quantitative understanding of Ca and P dynamics in the layer. This may help to evaluate various management options to reduce the excretion of unutilized P whilst maintaining bone integrity. Clearly, most of the eggshell is formed during hours when Ca absorption from the gut is relatively small. Management options may therefore focus on increasing Ca absorption from the gut during those hours that Ca is required, in order to decrease P losses and enable reduction of dietary P contents. Such strategies include increase of dietary Ca content, especially in the hours immediately before the light is switched off, increase of the length of the light period and use of a coarse Ca source to increase the retention time in the stomachs and thus increase Ca absorption during the night. An example of the latter strategy is presented in Table 9.3, assuming a doubling of the stomachs' retention time from 1.1 to 2.2 h with normal and coarse Ca, respectively.

The longer retention time results in improved P deposition in bone and excretion of unutilized P in urine. The net P deposition is improved for every single time of oviposition, except for oviposition at 7 h. On this day, no eggshell is formed during the night hours (Hours 16 to 24) and the layer does not benefit from an increased Ca supply in these hours, whereas the slower Ca release during the light hours until oviposition reduces Ca availability for eggshell formation

Table 9.3. Simulated averaged P flow in a 100-day period and simulated daily P flows (all in mg/day) in layers at various oviposition times after the light is switched on. The birds were fed coarse Ca.

Oviposition (h)	Days/ 100 days	Absorbed	Maintenance	Into egg	Into bone	Not utilized
1	15	311.1	23.8	111.6	122.7	53.0
2	16	311.1	23.8	111.6	85.0	90.7
3	20	311.1	23.8	111.6	29.8	145.9
4	17	311.1	23.8	111.6	–23.7	199.4
5	12	311.1	23.8	111.6	–74.7	250.4
6	10	311.1	23.8	111.6	–122.7	298.4
7	5	277.2	23.8	101.4	83.0	69.0
Pause day	5	277.2	23.8	111.3	34.2	107.9
Weighted average		307.7	23.8	111.1	18.6	154.3

in those hours. Overall, excretion of unutilized P is reduced from 221.1 to 154.3 mg/day. Further reductions are possible since bone P balance is positive and dietary P content may be reduced. The model developed is a tool to quantify Ca and P dynamics within a 24 h period based on an understanding of the processes involved and to evaluate Ca and P dynamics for a wide range of oviposition times. Thus, the model will help to evaluate feeding strategies aimed at reducing P excretion to the environment in poultry manure.

Conclusion

Layers exhibit a complex mechanism to maintain homeostasis in various nutritional conditions aimed at a proper distribution of available Ca over several metabolic processes. Calcium is stored almost entirely as hydroxyapatite crystals of Ca phosphate and Ca dynamics are thus closely related to those of P. An inadequate supply of Ca or P may lead to excessive withdrawal of Ca phosphate from the medullary bones and make these bones weak. An optimal diurnal supply of Ca to hens with varying moments of oviposition will help to reduce excretion of P in poultry manure. A mechanistic model of Ca and P metabolism has been developed that helps to understand the interaction between these minerals. The model is used to evaluate strategies to maintain bone integrity and reduce the wasting of dietary P. Further *in vivo* studies are needed to evaluate the sensitivity of the model settings and help to improve eggshell quality in the next decade.

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10 Efficiency of Phosphorus and Calcium Utilization in Dairy Cattle and Implications for the Environment

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Introduction

Phosphorus (P) is an essential element involved in numerous metabolic processes in the body of cattle. Efficiency of P utilization (the ratio of P excreted in milk to dietary P intake) is highly variable but in dairy cattle it is usually about 0.30 (Wu *et al*., 2000). The excess of P may be detrimental to the environment. Of particular concern is the amount of P surplus per hectare and the limited potential of soils to further accumulate P. Some soils are saturated with P as a result of intensive fertilization for many decades. Hence, a considerable fraction of manure P may leach from soils and contributes to water eutrophication. Legislation on P management is, or may become, a more important constraint on farming practice than legislation on N management (e.g. EU Water Framework Directive, http://eur-lex.europa.eu). For this reason, it is expected that the efficiency of utilization of P resources in intensive dairy farming has to increase substantially in the near future.

Besides environmental constraints, there are also economic or political arguments for improving P efficiency on dairy farms. World P resources for artificial fertilizer are becoming scarce and may become more expensive at a rapid rate (Cordell *et al*., 2009). There is no atmospheric loss of manure P such as for manure nitrogen (N). However, atmospheric N can be bound again by root systems of certain plant species and enrich soil N. Also, atmospheric N is used to produce artificial N fertilizer. Such recycling is not possible for P, which to a large extent is permanently lost from watersheds and rivers eventually to oceans. For this reason, P is lost much more permanently than N.

Scarcity of P stocks and more stringent future legislation to preserve water quality may lead to an increased pressure to maximize P utilization by cows and minimize P losses from manure. This can be achieved by lowering farm P import

 Phosphorus and Calcium Utilization and Requirements in Farm Animals (D.M.S.S. Vitti and E. Kebreab)

from purchased feeds and artificial fertilizers, and by making more efficient use of feed and manure P. A more efficient use of feed P can be achieved by reducing the amount of P ingested by dairy cows per unit milk yield. This chapter discusses the implications of nutrition on the efficiency of P utilization by cows, and puts this in the perspective of P balance at a farm level. To a lesser extent, efficiency of calcium (Ca) utilization is also discussed because the vast majority of P and Ca are stored in bones in an almost constant ratio of 1:2. We have addressed Ca as required for proper P utilization but not from a perspective of milk fever in early lactation.

Phosphorus: Nutrition, Requirements and Balance

Phosphorus nutrition balance and efficiency of utilization

Different intensities, different management options, different soil P status and different geographical situations may lead to a wide range of dietary P contents and amounts of P offered to dairy cows. Table 10.1 shows some typical P and N contents for frequently used roughages, concentrate ingredients and by-products,

Table 10.1. The P and N content (g/kg DM) of commonly used roughages, concentrate ingredients and by-products, in descending order of their net energy value for lactation.[a]

Product	Phosphorus content	Nitrogen content	DVE[b] (g/kg DM)	NEL[a] (MJ/kg DM)
Vegetable oil	0	0	0	24.4
Maize	3.1	14.9	117	8.7
Wheat	3.6	20.7	113	8.2
Peas	4.6	39.2	123	8.2
Rumen-resistant soybean meal	6.5	83.7	424	7.9
Maize gluten feed	10.1	38.1	105	7.5
Palm kernel expeller	6.1	26.3	116	7.4
Beet pulp	0.7	17.8	106	7.1
Tapioca	0.8	4.5	85	7.1
Soybean hulls	1.5	20.4	85	7.0
Grass herbage	4.3	36.0	96	6.9
Pressed potato fibre	1.8	11.5	92	6.8
Rumen-resistant rapeseed meal	12.5	60.8	295	6.5
Wet brewers' grains	6.2	39.8	138	6.5
Maize silage	2.0	13.0	51	6.4
Grass silage	4.2	31.0	67	6.1
Sunflower seed meal	13.3	62.9	139	6.0
Cane molasses	0.7	9.5	68	5.9

[a]Net energy for lactation according to the Dutch energy evaluation system VEM for dairy cattle (Van Es, 1975; CVB, 2007).
[b]Intestinal digestible protein values according to the Dutch protein evaluation system, the DVE/OEB system 2007 for dairy cattle (CVB, 2007, revision of Tamminga *et al.*, 1994).

although within every dietary component there may be substantial variation in N and P content, depending on origin or farm management.

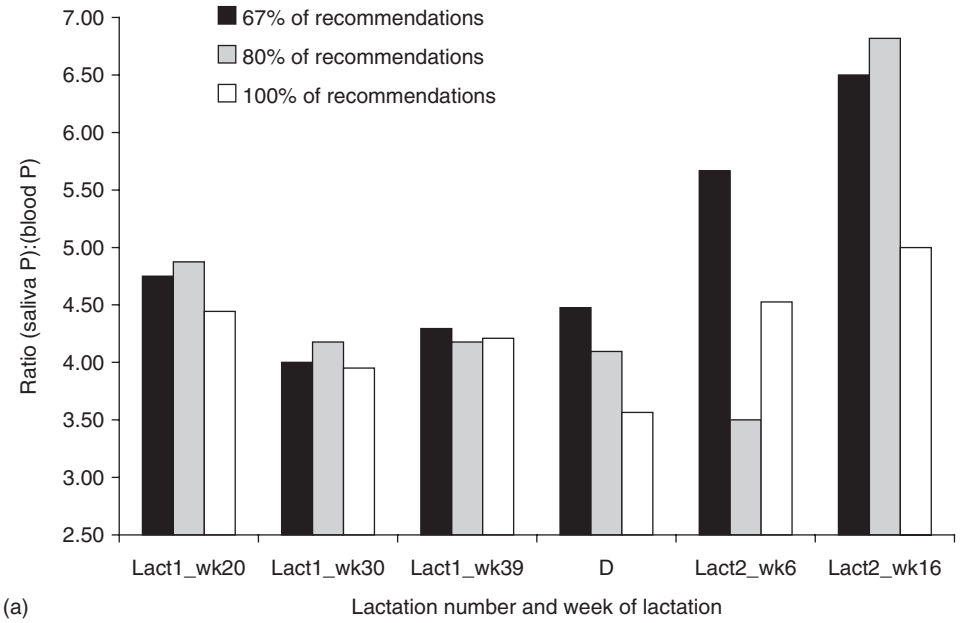
The type of diet strongly affects the amount of P ingested and of P excreted by dairy cows. With a simplified calculation method of the P balance of a dairy cow, which includes P ingestion, P retention with milk and P excretion with faeces, some rough calculations can be made to demonstrate sensitivity of efficiency of P utilization for milk synthesis and of P excretion for dietary changes. These calculations were made assuming that no P is added to the diet with premixes, and that P excretion with urine, as well as mobilization or deposition of P to bone and other body storages (including P deposition in the uterus of pregnant animals), is negligible. Changing the composition of a diet for a dairy cow producing 30 kg/day of milk from a grass silage diet supplemented with protein-poor concentrates to a maize silage diet supplemented with protein-poor by-products (hence minimizing protein allowance) indicates the maximum increase of P retained in milk when expressed as a percentage of P ingested. Retention of P in milk increases from 34 to 60% (relative increase of 76%; Table 10.2), assuming that there are no detrimental effects of P depletion on digestion and milk yield. Accordingly, the percentage of ingested P excreted in faeces decreases from 66 to 40% (a relative decrease of 40%). These results are in line with observations and predictions made by Kebreab *et al*. (2008), who studied P pollution in Ontario dairy cows and measures to reduce P pollution. At the same level of P intake as with diet 1 (Table 10.2), they predicted a value below 60%. With the simple balance calculations 66% is calculated, but Kebreab *et al*. (2008) assumed 40 instead of 30 kg of milk per day. Correcting for this difference in milk yield leads to almost identical outcomes of the percentage of P intake that is excreted in faeces. A similar comparison for the low level of P intake with diet 3 (Table 10.2) indicates that Kebreab *et al*. (2008) already predicted an identical value of 55% without correction for the difference in milk yield. After correction for difference in milk yield, predictions by Kebreab *et al*. (2008) are higher. Under conditions of low P intake the simple balance calculations adopted in this study (Table 10.2) hence deviate from outcomes with the dynamic model, which was indicated by the levelling off of the reduction in faecal P excretion with a progressive decrease in P intake (Kebreab *et al*., 2008). This result confirms that other mechanisms (including mobilization of P and secretion of P with saliva, Fig. 10.1a) become more prominent at low P intakes. Despite these differences at low levels of P intake, both methods clearly indicate that both efficiency of P utilization (retention in milk) and P excretion with faeces are particularly sensitive to the amount of P ingested.

The order of magnitude of the outcomes in Table 10.2 is thought to be realistic because the influence of the daily amount of P mobilized or deposited in the cow's body was in some studies around 10% of the daily amount of P ingested, and did not seem to be dependent on lactation stage, even at low P intake (Wu and Satter, 2000; Valk *et al*., 2002). With a P allowance to lactating cows of only 67% of previous Dutch recommendations (about 2.5 g P/kg dry matter and about the lowest P content tested in dairy cows for prolonged periods; see Table 7.13 in Pfeffer *et al*., 2005), Valk *et al*. (2002) measured at 20 weeks in the first lactation and at 16 weeks in the second lactation a net mobilization from the

Table 10.2. Calculated P balances[a] for four diets of different composition assuming a daily milk yield of 30 kg (containing 1.0 g P/kg of milk) and feed intake based on the Dutch net energy system for lactation (CVB, 2005).

Dietary component	Proportion % DM	P content g/kg DM	P intake g/day	Recommended[b] g P/day	Milk P		Excreted P	
					g/day	% P intake	g/day	% P intake
Diet 1		4.3	87	61	30	34	57	66
Grass silage	75	4.2						
Concentrates	25	4.8						
Diet 2		3.9	77	61	30	39	47	61
Grass silage	40	4.2						
Maize silage	35	2.1						
Standard concentrates	25	5.6						
Diet 3		3.3	67	61	30	45	37	55
Maize silage	75	2.1						
Protein-rich concentrates	25	7.0						
Diet 4		2.5	50	61	30	60	20	40
Maize silage	75	2.1			if P is not limiting milk yield			
By-products	25	3.5						

[a]Excreted P (g P/day) = P intake (g P/day) – P retained in milk (1.0 g P/kg milk × 30 kg milk/day).
[b]P recommendations according to CVB (2005).



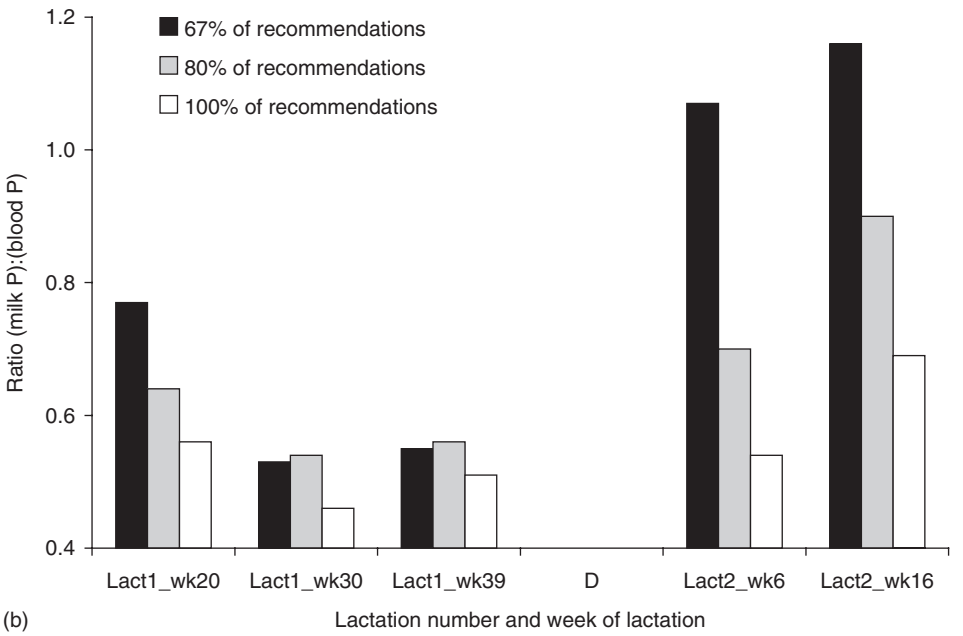


Fig. 10.1. (a) Ratio of saliva P concentration to blood P concentration, and (b) ratio of milk P concentration to blood P concentration in dairy cows fed at either 67% (■), 80% (▒) or 100% (□) of P recommendations (previous to the renewed recommendations by CVB, 2005) during two consecutive lactations. Observations were made at lactation week 20 (Lact1_wk20), 30 (Lact1_wk30) and 39 (Lact1_wk39), during the subsequent dry period (D), and during the second lactation at lactation week 6 (Lact2_wk6) and 16 (Lact2_wk16). Data were derived from Valk *et al.* (2002).

body of 13% and 2% of P intake, respectively. However, at 30 weeks and 38 weeks in the first lactation, but at 6 weeks in the second lactation, this mobilization appeared to be zero. In contrast, in a recent study by Taylor *et al*. (2009), much higher mobilization of 25% of P ingestion was reported during the first two weeks of lactation. The effects of various levels of dietary Ca on bone mobilization were evaluated and it might be expected this would have affected P mobilization as well. Nevertheless, P mobilization seemed unaffected and was invariably high for all Ca treatments. A similar high P mobilization was obtained by Knowlton and Herbein (2002), who fed cows diets containing 3.4, 5.1 or 6.7 g P/kg DM and measured P dynamics at week 3, 5, 7, 9 and 11 of lactation. The data indicated an interaction between diet and lactation week, meaning that dietary P concentration affects the duration of bone P mobilization. Cows in all treatment groups mobilized P from body reserves at week 3 of lactation, but only cows fed the low P diet remained in negative P balance during week 5. These results suggest that mobilization of P is stimulated with a low P intake and a high drain of P to milk. However, results of P balance vary with respect to the size of negative P balance and the moment during lactation when it occurs. Hence, P mobilization is not solely the result of P intake and stage of lactation. Other factors (historical effects, factors that affect long-term regulation, cow age or parity) are probably important as well. Urinary P excretion is usually less than 5% of total P in excreta, in particular with a low P intake. Significant amounts of P in urine only occur when plasma P concentrations exceed a threshold (Challa *et al*., 1989).

Because of the (temporary) effects of P mobilization and negative P balance, assumptions of the simple balance calculations may not exactly match the actual situation in the dairy cow. Transient changes in mobilization or deposition of P are important when comparing various studies on P metabolism with a different set-up and with observations made at different stages of lactation. Such temporary effects are of much less significance when considering efficiency of P utilization for a full lactation cycle (for example, when analysing P balance at farm level; Aarts *et al*., 2000). Still, they are important when analysing the effect of nutrition on the short- and long-term P and health status of the dairy cow.

Notwithstanding the importance of such temporary effects, results demonstrate that improvement of efficiency of P utilization can be accomplished by two means: first, by reducing P content of the diet, and, second by increasing the conversion of feed to milk. This means that both these aspects need to be addressed in particular when aiming to control P utilization by dairy herds.

Aspects of phosphorus requirements

Phosphorus for rumen microbial activity

Nutrients generally identified as limiting for microbial activity and growth in *in vivo* studies of rumen fermentation and in models quantifying this (e.g. France *et al*., 1982; Baldwin *et al*., 1987; Dijkstra *et al*., 1992) involve various types of carbohydrates, protein and ammonia. No representations are known for P, apart from the study of Kebreab *et al*. (2004) and later work of Hill *et al*. (2008). These modelling efforts did not explicitly include a representation of P limitation of

microbial growth. Although *in vitro* rumen P concentration below 0.5 mmol P/l limited microbial activity and cellulose degradation in particular (Komisarczuk-Bony and Durand, 1991), *in vivo* rumen P concentrations usually remain much higher than this value. Indeed, even with a P intake of 67% of previous Dutch recommendations for prolonged periods (Valk *et al*., 2002), saliva P concentrations were low as a result of low plasma P concentrations, but remained about 5 mmol P/l. This concentration is still ten times higher than the value established as critical *in vitro*. Three arguments can be given why rumen P concentrations will not become limiting (apart from extreme feeding conditions). First, saliva production contributes to the turnover of rumen fluid about three times more than drinking water. Rumen P fluid will hence reflect that of saliva P to a certain extent. Second, although the microbial population may at maximum incorporate about 70 g P/day (20 g P/kg microbial DM, 3.5 kg of microbial DM outflow to duodenum), comparable amounts of P are flowing in with saliva as well as ingested feed. Third, lower recommendations than the 67% tested by Valk *et al*. (2002) are not to be expected, and practically almost impossible to achieve unless all roughage consists of low-P sources such as maize silage (Table 10.2). It is concluded that P does not limit microbial activity in the rumen of lactating cows under practical feeding conditions of intensive dairy farming. This conclusion makes the fact that P depletion is often associated with a reduced feed intake (Kincaid and Rodehutscord, 2005) puzzling. It is questionable, however, if this is caused by detrimental effects of P depletion in the rumen, and it might be more related to other digestive or metabolic effects, or be associated with the type of diets involved with P depletion. Valk *et al*. (2002) did not observe a reduced DM intake with a continuous feeding at 67% of previous Dutch P recommendations (roughly 2.5 g P/kg DM) until a full lactation had passed (Valk and Šebek, 1999). In the second lactation DM intake was numerically lower by 10%, but this remained statistically insignificant. Other studies with higher levels of dietary P tested did not show an effect of dietary P content on DM intake (e.g. various studies reviewed by Pfeffer *et al*., 2005).

Phosphorus recycling with saliva

An increasing fraction of absorbed N is recycled to the gut when N intake is reduced (Reynolds and Kristensen, 2008). Excess absorbed N that is not retained by the cow is predominantly excreted with urine. Similarly, extensive recycling of blood P to the gut occurs and this may be of a similar magnitude to the amount of P absorbed when P intake becomes low. In contrast with N, even with excessive amounts of P absorbed, almost all P that is not retained in milk or in the cow body is recycled to the rumen via saliva. This makes the dairy cow very efficient in preserving ingested P for cow metabolism and rumen microbial activity. Saliva P concentrations reflect blood P concentrations (see Chapter 7 for details). Hence, if a relatively smaller fraction of absorbed P remains available in the blood pool, less P is also available for recycling with saliva to the rumen. Conceptually, this is similar to urea recycling to the rumen (Dijkstra *et al*., 1992). Valk *et al*. (2002) indeed established a clear relationship between saliva P concentrations and blood P concentration. Based on this, Hill *et al*. (2008) assumed P recycling to be proportional to blood P concentration and saliva fluid flow rate.

It is not clear, however, whether this recycling is further stimulated when P concentrations are drastically lowered. The salivary gland strongly concentrates P in secreted saliva, which is an active process (Breves and Schröder, 1991). In lactating dairy cows a fivefold higher P concentration in saliva than in blood plasma was observed (Valk *et al*., 2002). It remains to be investigated, however, how dietary effects on saliva production rate and blood P (and Ca, which will be discussed later) concentrations affect the regulatory mechanisms involved. This regulation is an outcome of the interaction between P absorption from the intestine, P resorption from bone or soft tissues and P recycling with saliva (Fig. 10.2). Data from Valk *et al*. (2002) are interesting in this respect. During the first lactation the ratio of saliva P concentration to blood P concentration with the treatment of 67% was 1–7% higher than with the treatment of 100% of previous Dutch P recommendations (ratio values ranging from 4 to 5; Fig. 10.1a). However, this seems to have changed in the subsequent dry period and the second lactation when these ratios were 25 to 26% higher. This is an indication that feeding low dietary P levels may have large implications for P metabolism in the long term. This is indeed suggested by observations by Valk *et al*. (1999) of clinical effects (haemoglobinuria) and impaired feed intake and milk yield, and a low lactose content of milk.

Absorbed P that is subsequently recycled to the gut via the blood pool may have a feed origin or may be mobilized from the cow's body. This may involve rapid mobilization of P from soft tissues or bone, with regulatory mechanisms that operate more on a long-term basis (Vitti *et al*., 2000; Dias *et al*., 2006). The maximum amount of P mobilized relative to P intake seems to be larger than that of N relative to N intake during early lactation (Firkins and Reynolds, 2005; Van Knegsel *et al*., 2007).

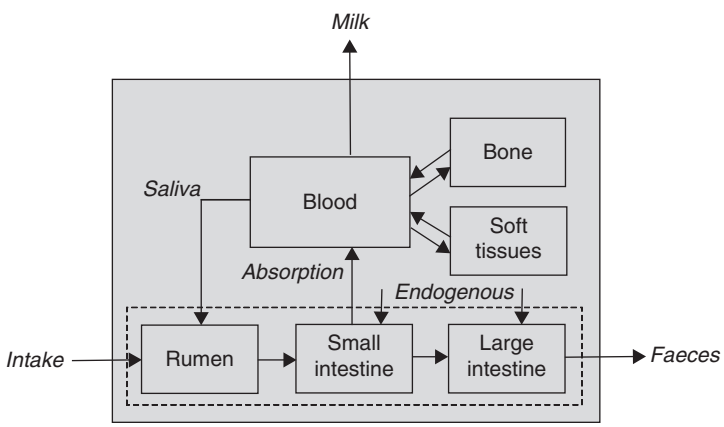


Fig. 10.2. Schematic representation of P flows in the dairy cow. Words in italics outside the grey box indicate the P inflow and P outflow from the cow's body, and were used in the simple P balance calculation discussed in the text. Words in boxes inside the grey box indicate pool sizes of P in the various metabolic or physical compartments of the gut lumen or the body of the dairy cow that are involved with the dynamics of P flows and pool sizes.

Although it seems that low P recommendations do not easily result in impaired rumen function, they may still have a detrimental effect on P homeostasis and cow performance in the long term. Maintaining P homeostasis means maintaining blood P concentration as well as regulating the size of P stores in the cow's body. A better understanding is needed of their interactions and their dynamics before applying dietary P contents below current recommendations.

Phosphorus absorption

Absorbability of P in various feedstuffs may be considered an important determinant for efficiency of P utilization by dairy cows. Because of rumen microbial activity, the majority of P flowing into the intestine is in a different form from that of feed P ingested. With the high phosphatase activity of the rumen microbial population, most phytic acid P in feed is converted into inorganic phosphate. Part of this is incorporated into microbial P, which is for about 80% P in nucleic acids and 10% P in phospholipids (Kincaid and Rodehutscord, 2005). The P fraction included in rumen-resistant feed protein probably escapes phosphatase activity (Park *et al*., 1999). Saliva delivers a major inflow of inorganic phosphate to the rumen in addition to feed P. Considering these processes, several P fractions flowing to the intestine have been identified (Kebreab *et al*., 2004; Hill *et al*., 2008): inorganic P, organic P (including P in phytic acid, which is undegradable by intestinal phosphatases, P in degradation products of phytic acid, which can be degraded subsequently by intestinal phosphatases, and other forms of organic feed P) and microbial P (mainly nucleic acid P).

For monogastric animals, it is well established that dietary manipulations and conditions in the lumen of stomach and duodenum affect phytase activity and the degradation rate of phytic acid. The availability of P in phytic acid for the cow might depend on the activity of intrinsic plant phytase, on the effect of rumen pH on microbial phytase activity and on its retention time in the rumen. Illustrative is the recent finding by Knowlton *et al*. (2007) that with a combination of exogenous phytase and cellulase added to the diet apparent faecal P digestion improved from 40 to 46%. However, the effect was evaluated with a rather high dietary P content of 4.2 g/kg DM, whereas it would be most interesting to know the effect with low P intake. Phytic acid is known to form complexes with proteins at low pH values such as those found in the abomasum, which are thought to affect intestinal protein digestion in monogastric animals. To complicate this further, the dietary content of Ca and Mg, type of protein, proteolytic enzymes and pH may all affect this complex formation. Also complexes are formed with divalent cations such as Ca, which might affect Ca and perhaps even P absorbability. However, it is not certain whether such interactions, as described for monogastric animals, are of significance for explaining P absorption (and perhaps even protein digestion) in the intestine of dairy cows.

Most feed P is eventually degraded into the form of inorganic P during digestion by ruminants and mainly inorganic P is excreted with faeces (Dou *et al*., 2000; Toor *et al*., 2005). Hence, it is degradable and absorbable in principle. Incidentally, inorganic P is more readily available for plant growth and algal growth in surface water than organic P. The microbial population in the rumen has a very high phytase activity, which makes the activity of intrinsic phytase in

feed less relevant for explaining P absorbability. As a result of this high phytase activity, variation in P absorbability among various feedstuffs is small (Pfeffer *et al*., 2005) and at least much smaller than experienced with monogastric animals. Phytic acid P is the main fraction in dietary organic P. Perhaps a better qualification for absorbability as an intrinsic feed characteristic would be the term degradability, because rumen degradation of unabsorbable phytic acid into an absorbable form mainly determines the absorbability of this P fraction in the small intestine. The foregoing means that evaluation of the variability of P absorbability of feeds should focus on three flows contributing to intestinal P: (i) rumen-degraded organic feed P not incorporated into microbial mass; (ii) rumen-degraded organic feed P incorporated into microbial mass; and (iii) rumen-resistant organic feed P. All three flows strongly depend on nutritional strategy and microbial activity in the rumen. Evaluating variation of these P flows hence involves the evaluation of nutritional strategy in all its aspects. Quantitative aspects of rumen function were reviewed previously (Dijkstra *et al*., 1992, 2002). Suffice it to say that the amount of organic matter fermented is the strongest determinant of quantity of microbial matter flowing to the duodenum. Furthermore, the efficiency of microbial crude protein synthesis (including P-containing nucleic acids) from fermented organic matter depends on many factors (feed intake level, roughage:concentrate ratio, chemical composition of dietary DM, intrinsic degradation characteristics of these chemical fractions and fractional passage rate of rumen contents).

For microbial crude protein (including nucleic acids) an intestinal digestibility of 85% is assumed (Tamminga *et al*., 1994), and this is presumed to be applicable to microbial P as well (Hill *et al*., 2008). The digestibility of rumen-resistant feed proteins is high for feeds with the highest digestible protein value (Table 10.1). This led Pfeffer *et al*. (2005) to conclude that the potential absorption efficiency is very high and that a lack of influence of the source of feed P on absorbability is mainly caused by the intensive microbial phytase or phosphatase activity in the rumen. This implies that efficiency of P utilization in cows is more sensitive to level of P intake, rumen fermentation and milk yield than to slight differences in absorbability of P in rumen-resistant feed protein sources. If P allowance is very low, the sensitivity to differences in absorbability could increase. However, low P allowance often coincides with low protein supplementation and in that case small amounts of rumen-resistant feed protein and associated organic P are being fed. Hill *et al*. (2008) performed model simulations and indeed concluded that P intake had a larger impact on faecal P excretion than the type of feed P. Furthermore, faecal P excretion was not very sensitive to rumen phosphatase activity. Finally, intestinal P absorption was an important site of regulation in combination with the P dynamics of soft tissues and bone. The latter seems to be in contrast with our conclusion that P absorbability, as a feed characteristic, is not that important. This is not the case. The point made is that P absorbability is not mainly determined by some intrinsic feed characteristic, but that it is regulated at the level of the intestine as a digestive organ. The conclusions of Hill *et al*. (2008) were the outcome of the relationships adopted for the diminishing effect of increasing P concentrations in blood on P absorption from the intestine, for which they referred to findings by Hibbs and Conrad (1983). Also, Schröder

et al. (1995) established in isolated sheets of the small intestine of sheep adapted to P depletion that active transport of inorganic phosphate was strongly stimulated. These findings are in support of the concept that blood P concentrations affect absorption of P from the intestine.

The implication of the foregoing discussion is that the dynamics of blood P concentrations and associated regulatory responses in the dairy cow probably deserve more attention than simply absorbability as an intrinsic feed characteristic comparable to feeding values mentioned in feeding tables. This particularly holds for situations in which relatively low P intake is combined with a high milk yield. This makes the situation for P different from that of N.

Inevitable and endogenous phosphorus loss

A first determinant of P requirement is the inevitable P loss by the dairy cow. A large fraction of ingested P is transformed into P incorporated into microbial matter in the rumen. Part of this microbial P remains unavailable for the dairy cow. The majority of microbial P (Kincaid and Rodehutscord, 2005) is present in nucleic acids (about 80%) and phospholipids (about 10%). If digestibility of microbial P is indeed of the same order as that of microbial N (roughly 85%; Tamminga *et al*., 1994), then loss of microbial P would contribute substantially to inevitable P losses by the dairy cow. With 15% undigested microbial P and 70 g microbial P per day flowing to the duodenum, this inevitable loss would be 10 g P/day, which is of a similar order of magnitude to the negative P balances measured in cows. It also implies that all nutritional factors that affect microbial growth will affect microbial P flow to the intestine as well. When this is combined with variation in the P content of the diet, large variation seems possible in the form of P available for intestinal digestion by the dairy cow.

Besides the inevitable P losses, there are endogenous losses of P of cow origin, comparable to the concept used for endogenous N loss in protein evaluation systems. The sloughing of intestinal epithelial cells is thought to be the major cause of this P loss (AFRC, 1991) because intestinal secretions appear to contain relatively small amounts of P in contrast to saliva flowing into the reticulo-rumen. A similar concept is used in protein evaluation systems (Tamminga *et al*., 1994) and endogenous loss is assumed to be related to the amount of (undigested) dry matter (DM) flowing through the intestinal tract. However, such a presumption for the case of endogenous P loss is questioned by Pfeffer *et al*. (2005). They suggested that the digestible part of ingested DM affects endogenous P secretion instead of the indigestible part as assumed with endogenous N secretion. There is still uncertainty about the mechanism involved with endogenous P losses. Perhaps the term loss is deceiving in this respect if endogenous P is more related to functional metabolism (Pfeffer *et al*., 2005) instead of simply a loss of P with sloughed gut wall or secreted material.

Milk phosphorus

Besides inevitable P losses, milk P is the major determinant of P requirement of high-yielding dairy cows. Often, a P content of milk of about 0.9 g P/kg milk is mentioned. However, variation within and between experiments seems to be large, with values ranging from 0.7 to 1.1 g P/kg milk (Pfeffer *et al*., 2005), which

is considerable. Considering the fraction of ingested P retained as milk P, such variation contributes significantly to variation in the apparent efficiency of P utilization. According to the simple balance calculation, the efficiency of P retention in milk for a dairy cow consuming 60 g of P/day (20 kg DM/day, 3.0 g/kg DM) and producing 25 kg of milk/day differs by 20% when average milk P content changes by ±0.2 g P/kg milk (Fig. 10.3). Therefore, variation in milk P content seems to deserve attention when studying variation in efficiency of P utilization by dairy cows. Figure 10.3 demonstrates results from a farm monitoring project (Cows and Opportunities; Šebek *et al*., unpublished) in which milk P concentrations were analysed on 16 farms that differed in soil type, management and composition of dairy rations. Similar to milk P results reviewed by Pfeffer *et al*. (2005), a wide range of values becomes apparent (from 0.82 to 1.11 g P/kg milk) (Fig. 10.3). This variation remains hard to explain from a farm management perspective. Also, there were surprising changes in milk P content in five or six successive analyses of bulk tank milk delivery during 3 weeks. Milk P content at single farms at various deliveries frequently varied by 0.15 g P/kg milk, which is about 16% of the overall average milk P content of 0.94 g P/kg milk for these farms. Such variation has a dramatic impact on efficiency of P utilization by cows. Nutritional control of milk P content is unknown, although there are some indications that it is slightly related to milk protein content. Wu *et al*. (2000) observed an increase of 0.015 g P/kg milk with an increase of 1 g protein/kg milk. The P concentration in milk is far higher than P concentration in blood

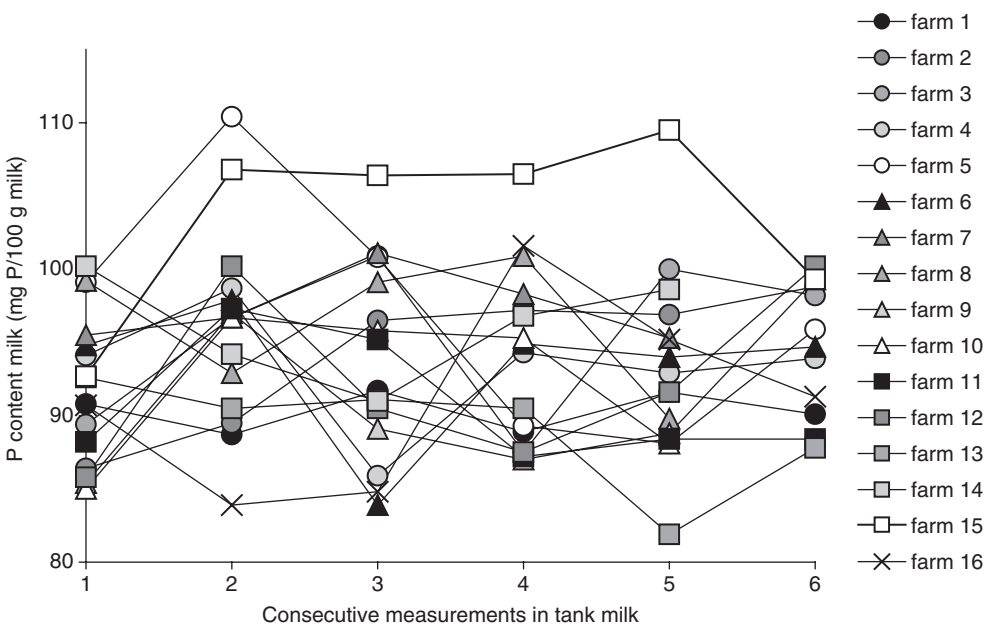


Fig. 10.3. Variation in milk P content in five or six consecutive measurements of P content in tank milk delivery in October and November 2006 on 16 farms intensively monitored in the project Cows and Opportunities (Adapted from Šebek *et al*., unpublished).

plasma and P (and Ca) seems to be actively transported or secreted into milk, comparable to proteins and lactose (Neville and Peaker, 1979).

Also long-term P metabolism studies in cows (Valk *et al*., 2002) indicate that milk P content varies. Reducing P allowance from 100% to 67% of previous Dutch P recommendations during two subsequent lactations caused the ratio of milk P concentration to blood plasma P concentration to increase on average by 20% in the first lactation, but 83% in the second lactation (Fig. 10.1b). Although part of this difference can probably be attributed to sampling at a later stage during the first lactation, a large difference with the second lactation becomes apparent. Such long-term effects may be interpreted as indication of interference of dynamics of P flows from body P storages with regulation of blood P in the long term (over full lactations). Only multiparous cows were used in this particular study and hence effects of ageing on P metabolism have probably remained small but cannot be ruled out. Confounding of observed defects in P metabolism with ageing is probably much more prominent when heifers are studied.

Because milk is the largest drain of absorbed P in the dairy cow, the presumption of constant milk P content when analysing strategies to optimize efficiency of P utilization may be questioned. Efforts are needed to investigate the regulation of milk P content (assuming that it is regulated), and to find a method to take into account or anticipate this variation in milk P. Current models of P metabolism do not take into account such variability in milk P content. Moreover, in current P recommendations and legislation, P content of milk is assumed not to vary. It is not exactly clear what factors determine P content in milk but its investigation seems worthwhile, considering the impact on efficiency of P utilization by dairy cows.

Phosphorus in bone and soft tissues

From the point of view of cow health and bone quality, the transient response of P dynamics in bone and soft tissues merits further research. The transient response may be rather slow for bone P, but it is much faster for soft tissue P (Vitti *et al*., 2000; Dias *et al*., 2006). With a low P allowance, the depletion of P in soft tissue may not be followed instantaneously by the resorption of bone. Understanding how to prevent negative effects on cow health by short-term depletion of soft tissues and by long-term P depletion of bone probably requires further insight into the interplay between the drainage of blood P to milk and saliva, P absorption by the intestine and P mobilization from bone and soft tissues. This interplay is also important when momentary observations of faecal P concentration are to be used as indicators of efficiency of P utilization by the cow (Kebreab *et al*., 2008). In contrast to assumptions in simple balance calculations, the transient responses of bone and soft tissues interfere with instantaneous values of faecal P excretion and may partly obscure the efficiency of P utilization or the P status of the cow.

Representing dynamic aspects

In the previous section details were discussed on various aspects relevant for P metabolism in the dairy cow. It appeared that P storage in soft tissues and bone

has a particularly important role in maintaining P homeostasis and blood P concentrations with low dietary P content. In this respect, P metabolism differs from N metabolism, which seems to rely much less on N storage in the body. For example, mobilization of N during early lactation is less than 2% of N intake, in the first few weeks after parturition only (Firkins and Reynolds, 2005). Mobilization of P may be much more prolonged although it seems to remain of a similar magnitude to the percentage of N intake when dietary P content stays well above 3 g P/kg DM (Wu *et al*., 2000; Valk *et al*., 2002). With more drastic reduction of P content to less than 2.5 g P/kg DM (Valk *et al*., 2002), it seems possible that the percentage can incidentally increase to more than 10% further on in lactation. In contrast, other studies (Wu *et al*., 2000; Knowlton and Herbein, 2002) clearly demonstrated a mobilization of P immediately following parturition, with the lowest dietary P content just above 3 g/kg DM. Apparently, various factors determine the extent, rate and duration of P mobilization in dairy cows. For matching P recommendations as closely as possible to actual P requirements at a certain stage of lactation, such effects need to be understood (Taylor *et al*., 2009).

This means that the dynamics of (regulation of) rumen P degradation, P recycling with saliva, P absorption in the intestine, P retention in milk and P mobilization or P deposition in soft tissues and bone need to be quantified. Integrative approaches with dynamic mechanistic models have already been demonstrated to be very useful to explain rumen N dynamics (Bannink *et al*., 2006). Simulation results corresponded with a review by Firkins and Reynolds (2005), who concluded that synchronizing N and energy availability for rumen microorganisms and maintaining a positive rumen N balance are likely not improving the efficiency of microbial growth. For P, dynamic models have been published by Kebreab *et al*. (2004) and Hill *et al*. (2008) that help in explaining the dynamics of P metabolism. With these modelling efforts, P requirements should not be treated as a separate topic, as with current P recommendations (Wu *et al*., 2000; Valk *et al*., 2002; Pfeffer *et al*., 2005), but they should be integrated with the representation of other aspects, such as rumen function, intestinal digestion, nutrient delivery to the cow, nutrient delivery and utilization by the udder and excretion with urine and faeces. Comparable to the storage of energy in the cow's body and its mobilization during early lactation, the important role of P mobilization or deposition needs to be included in an integrative and dynamic manner. The representation of historic effects of P nutrition on P status and available P reserves might prove to be very important when attempting to offer a diet with a low P content. It is probably more important than in the case of N, where the N reserves have a less prominent role to play when feeding diets with a low N content compared with P reserves.

Phosphorus recommendations in practice

Various countries have adopted different systems of P recommendation for dairy cows (Fig. 10.4). Valk *et al*. (2000) stated that there is agreement only with regard to P need for milk production. This means either that large safety margins have been used during the development of some of these systems or that there is still

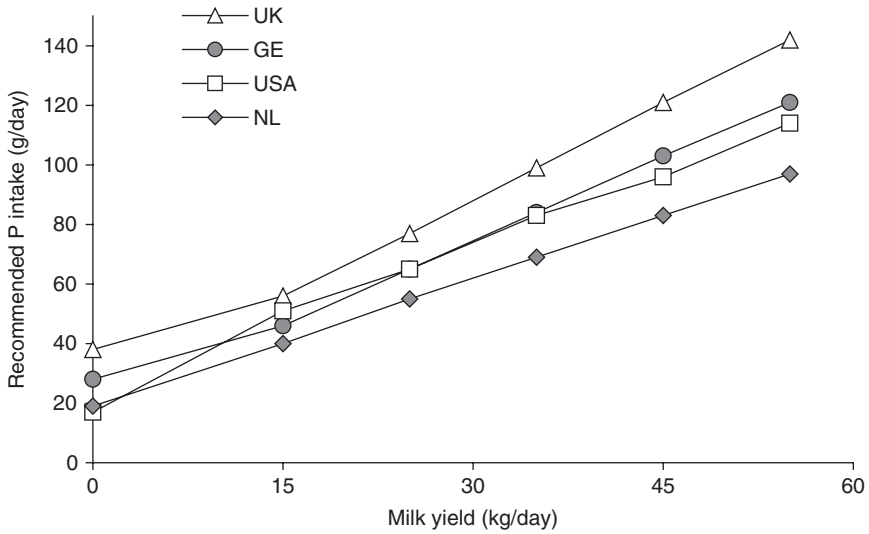


Fig. 10.4. Dependency of recommended P allowance on milk yield of (dry and lactating) cows in various countries: UK (AFRC, 1991); GE (GfE, 1993); USA (NRC, 2001); NL (CVB, 2005).

not much consensus about the actual P requirements of dry cows and lactating cows at various levels of milk production. From Fig. 10.4 it becomes clear that recommendations in the United Kingdom and Germany are highest and those in the Netherlands are lowest. With the aim to reduce P allowance, but to remain within safety margins of modern high-yielding dairy cows, various research groups have conducted *in vivo* experiments varying P allowance. In a 2-year study, Wu and Satter (2000) reported that reducing dietary P from 4.8 to 3.8 g/kg DM did not impair milk production or reproductive performance in dairy cows. Wu *et al*. (2000) showed that, over two to three lactations, feeding P at 3.1 g/kg DM to cows producing 12,000 kg per lactation decreased P concentration in bone, but the decrease was not severe enough to affect bone strength. Valk and Šebek (1999) performed experiments with feeding lactating cows at 100%, 80% and 67% of the previous Dutch P requirements (corresponding to 3.3, 2.8 and 2.4 g P/kg DM). Cows were followed during two lactations. They concluded that dietary P contents of 2.8 g P/kg DM are sufficient for cows producing 9000 kg of milk per lactation. The current Dutch recommendation is that P content should not be below 3.0 g P/kg DM. This seems to be a safe estimate because it was assumed that the coefficient of true P absorption is 70%, which is considered to be low (Pfeffer *et al*., 2005).

If the national differences in P recommendation reflect actual P levels in dietary DM, then large differences in faecal P excretion may be expected among countries. However, this is probably not the case. Despite the lowest amounts of P recommended in the Netherlands (Fig. 10.5), such low dietary P levels are not achieved with current grass-based diets because of high contents of P in grass

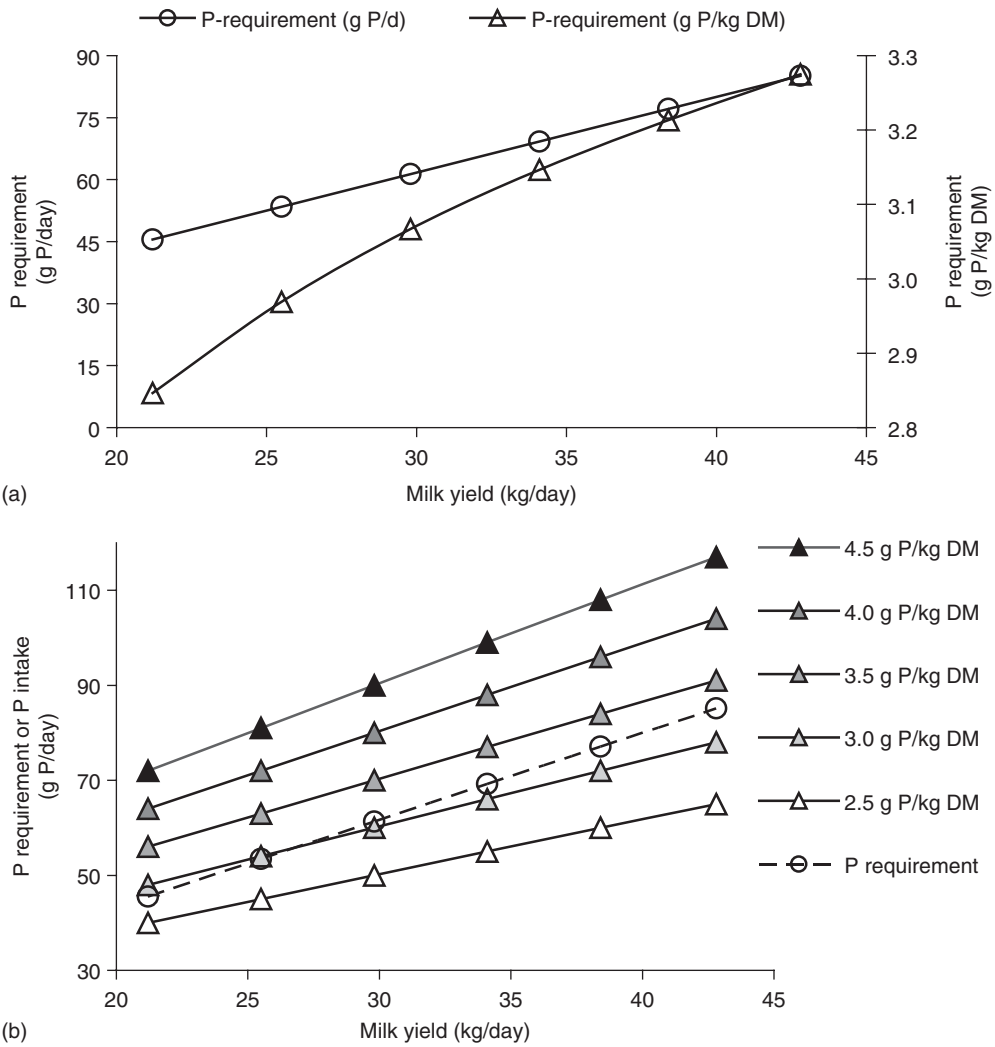


Fig. 10.5. Indication of the consequence of current Dutch P recommendations (CVB, 2005) with varying milk yield for (a) P requirement in g/day and g/kg DM, and (b) P requirement and P intake (both in g/day) with varying P content of dietary DM.

(Fig. 10.5). Extreme diets need to be formulated before a dietary P content of less than 3.0 g P/kg DM is reached (Table 10.2). From the perspective of fulfilling the P requirements of the dairy cow, this means that there is in principle much room left for improving efficiency of P utilization. Other reasons, such as the P status of soils and some agronomical reasons, mean that it cannot be fully used by the farmer. Purchase of P-poor concentrates only partly compensates for the high P content of grass. Only with a diet based on maize silage as the main roughage would such a strategy lead to a dietary P content close to the

Dutch P recommendations, and a high efficiency of P utilization (approaching about 60% with extremely low dietary P content; Table 10.2) and low P excretion in faeces.

Calcium Utilization and its Relationship with Phosphorus

Most P in the cow's body is stored in Ca–P complexes in bone. After parturition there is a strong Ca mobilization from bone in order to fulfil the high Ca requirements for milk production, and most cows in early lactation are in a negative Ca balance (Braithwaite, 1976). In response to hypocalcaemia, parathyroid hormone is secreted. This stimulates the conversion of 2,5-hydroxycholecalciferol to dihydrocholecalciferol, which increases bone resorption. Because the Ca:P ratio is higher in bone than in milk, during bone mobilization more P is mobilized than required for milk. Such an excess of P will be excreted in faeces during early lactation. This means that nutritional strategies to improve Ca metabolism, for example feeding acidifying diets (Block, 1994) to stimulate Ca resorption from bone after parturition, also affect P metabolism and excretion.

Calcium absorption from the gut (as a fraction of Ca intake) increases with increased Ca requirements, notably immediately after calving (Van't Klooster, 1976). In contrast to monogastric animals, intestinal absorption of Ca and P seems to be rather independent from each other in cows (Pfeffer *et al*., 2005). Excessive Ca concentrations do not negatively affect P absorption. Blood Ca concentration is under a much stronger hormonal control (Goff *et al*., 1991) than blood P. The Ca concentration in blood plasma is kept within a narrow range around a value of 5.5 mmol/l, whereas that of P has been shown to vary from 0.3 to 2.2 mmol/l in the long-term experiments of Valk *et al*. (2002). Taylor *et al*. (2009) studied the effect of three dietary Ca contents on Ca and P balance during the first 20 weeks of lactation. The Ca balance was only negative during the whole experimental period, except week 20, for the lowest dietary Ca content (5 g/kg DM). However, it appeared unrelated to P balance, which was negative during the whole period, without significant differences among the various Ca treatments tested. Pfeffer *et al*. (2005) suggested that uncoupling of P and Ca is a specific adaptation of ruminants that allows for a maximum absorption of P during periods of P depletion.

Blood P concentration is regulated at the site of P absorption, recycling and excretion in urine (with excess blood P). Nevertheless, changes in blood Ca concentrations are still probably guiding the resorption of bone at the onset of lactation and in this manner also blood P concentrations and availability. Because Ca dynamics seems to rule the regulatory mechanisms during early lactation, the dynamics of Ca metabolism needs to be taken into account when evaluating the effects of P intake on the dynamics of P metabolism. Although Ca metabolism has already been addressed in kinetic models describing outcomes of Ca isotope infusion studies (Vitti *et al*., 2000; Dias *et al*., 2006), it is not incorporated in dynamic models of P metabolism in ruminants (Kebreab *et al*., 2004; Hill *et al*., 2008).

Farm Phosphorus Balance

Many studies indicate that efficiency of P utilization by dairy herds has a significant influence on the impact of on-farm P losses to the environment. Aarts *et al*. (2000) investigated P surplus on a prototype experimental dairy farm on sandy soils in the Netherlands with a dietary P content of 3.5 g/kg DM. Environmental problems with intensive farming are most pronounced on this type of soil. By minimizing import of P with purchased feeds and without artificial P fertilizer, an average surplus of 4.5 kg P/ha was achieved from 1994 until 1997. However, under similar farming conditions, commercial farms realized between 1983 and 1986 a more than six times higher P surplus of 32 kg P/ha. Utilization of P by a dairy herd of an experimental farm was about 50% higher (30% of P imported) in comparison with that achieved by the commercial farms (22% of P imported), because P input was 35 kg P/ha less (20 kg feed P/ha less; 15 kg artificial fertilizer P/ha less). As a result, the size of reduction in P imported was almost equal to the decrease of total farm P surplus. Besides P utilization by the dairy herd, efficiency of utilization of manure P by crops also needs to be included among other aspects of farm management (Schröder *et al*., 2005). With a strongly decreased P input to soil, the percentage P recovered as crop P increased to about 90%. Although an integrated approach is needed to optimize P utilization on dairy farms, these results clearly demonstrate the importance of a reduced P import and an improved efficiency of P utilization by the dairy herd for minimizing whole-farm P surplus.

Various other studies indicate that feed import is the primary source of P input as a strong determinant of whole-farm P balance. Spears *et al*. (2003) investigated farm P balance of commercial dairy farms in Utah and Idaho and also concluded that increasing the conversion of feed P into P in milk and meat is important to reduce farm P surplus. On average, calculated import of feed P made up 85% of total farm P import. But variability among farms was extremely high and the calculated P surplus varied from –17% to 34 t of P/year (average herd size 466 cows and P surplus per cow of 10.7 kg/year). This means that feed P strongly affects the efficiency of P utilization in dairy operations. For example, Kebreab *et al*. (2008) calculated that reducing dietary P content by just 0.1 g/kg DM would save Ontario dairy operations enough P to satisfy the entire Ontario dairy herd for the whole dry period.

Higher milk yield per cow can also be associated with an improved conversion of feed P to milk P. But, if this higher milk yield per cow is realized with a higher P import in purchased feeds (concentrates, energy-rich by-products), the trade-off between a more efficient P utilization by the dairy herd and a higher P import to the farm needs to be taken into account. Finally, the choice between home-grown protein sources in the diet and import of protein-rich feeds also affects the level of P import to the farm. Commonly used protein sources (Table 10.1) sometimes have a high dietary P content and may cause substantial P import to the farm. Avoiding this without compromising dietary energy and protein value and milk yield will immediately contribute to an improved efficiency of P utilization by the dairy herd and on the whole-farm level. In most cases in current practice, there seems to be much room left for manipulating

dietary P content without any compromise with respect to feeding recommended amounts of P.

Conclusions

Several studies have already been performed on efficiency of P utilization by dairy cows with varying outcomes, maybe as a result of different P (and perhaps Ca) status of the cows involved, because of historic effects or because of differences in duration of these studies. Long-term trials with high-yielding cows and drastically lowered dietary P contents are scarce. Such studies are needed, however, to investigate critical P recommendations and to investigate the effects and importance of temporary effects of nutrition on P metabolism and P balance. The reason why such studies have not yet been performed on a wider scale is probably that with practical roughage-based diets it is not easy to formulate a diet with a P content below recommendations, unless maize silage is the main roughage component in the diet. Only when dietary P contents are drastically lowered could there be a serious need to investigate more thoroughly the impact of dietary P content on temporary and long-term P metabolism. Early lactation is a critical period because of the very high drain of P to milk in this period. Minimum P intake recommendations differ widely among countries, with the Netherlands currently adopting the lowest recommendations. Nevertheless, all recommendations are applied to highly similar herds of Holstein-Friesian cows. Further research needs to clarify more precisely which P recommendation is still justifiable under certain farming conditions or a certain physiological state of the cow. The case for P is very different from that for N. In contrast to N (and current energy and feed evaluation systems), with P attention should not be focused as much on determining P absorbability in the intestine, but should include the dynamic aspects of P metabolism and P storage in bone and soft tissues. Dynamic modelling seems useful when evaluating the dietary P contents in the lower range. The critical importance of Ca metabolism in periparturient dairy cows justifies its inclusion in such modelling efforts.

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Index

Page numbers in **bold** type refer to figures and tables.