# Modelling the phosphorus intake, digestion, utilisation and excretion in growing and finishing pigs

**By**

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**A thesis submitted for the degree of Doctor of Philosophy (PhD)**


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Submitted September 2014

# Abstract

The overall aim was to develop a model of Phosphorous (**P**) intake, digestion, utilisation and excretion in growing/finishing pigs, and use it to investigate the consequences of different P management strategies. Initially, a dynamic, deterministic model was developed (Chapter 2). It was able to predict the digestible (**digP**) requirements of pigs of different genotypes and stages of growth, as well as the consequences of different dietary contents of P, Calcium (**Ca**) and exogenous phytase. The model was also able to predict the excreted amounts of soluble and insoluble P. Subsequently (Chapter 3) the model was evaluated against independent data and a sensitivity analysis of its predictions to model parameters was undertaken. Model outputs were most sensitive to the values of the efficiency of digP utilization and the non-phytate P absorption coefficient from small intestine. The model predicted satisfactorily the quantitative pig responses, in terms of P digested, retained and excreted, to dietary variations. The model performed well with 'conventional', European feed ingredients and poorly with 'less conventional' ones, such as DDGS and canola meal. In Chapter 4 the model was converted into stochastic, by introducing variation between pig digP requirements and the consequences of two strategies were investigated (phase feeding and sorting). The former was more effective in reducing P excretion than the latter. Finally the model was extended to include uncertainty in feed composition (arising from variability in ingredient nutrient content and mixing efficiency) to investigate how this would affect the outputs of the model. Due to the assumptions made, uncertainty about feed ingredient composition contributed more to performance variation than uncertainty regarding mixing efficiency. When uncertainty about both feed composition and pig characteristics was considered, it was uncertainty about feed composition rather than pig genetic characteristics that proved to have the dominant influence on variability in pig performance.

## Declaration

This thesis has been composed by myself and has not been submitted as part of any previous application for a degree. All sources of information have been specifically acknowledged by means of referencing.

Vasilis Symeou

# Dedication

I dedicate this PhD thesis to the pig farmers. They face so many challenges, namely: (1) variable feed prices with an upward trend; (2) competitiveness from cheaper markets; and (3) higher welfare and environmental standards. And yet they keep on producing, they are still in business, evolving to be more efficient and even reinvest back into the business during profitable periods. This shows they love their job; they take pride in providing the society with high quality British pork, produced to high environmental and animal welfare standards. They should be admired!

# Acknowledgements

This PhD has been made possible only through the funding of the British Pig Executive (BPEX), for which I am very grateful.

I would like to thank Prof. Ilias Kyriazakis, my main supervisor, for dedicating so much time and effort in this thesis. His professional scientific advice and patience allowed me to become a critical researcher. Developing the model in the programming language Excel VBA (Visual Basic for Applications) would not have been possible without the help of Dr. Ilkka Leinonen and his determination to overcome difficult computational obstacles. I would also like to thank Prof. Sandra Edwards for being sensitive to my families' struggles and keeping my motivation high during the final stages of the PhD.

I cannot thank my wife Carolina enough, for her patience and understanding during my prolonged absence from Cyprus. Finally, I would like to thank my best friends Antonis Iaponas and Maria Korea, as well as my parent (Giorgos and Eleni) and my parents in law (Takis and Lyda) for their encouragement and support throughout my PhD.

# Publications and conference abstracts

## Peer-reviewed publications

Symeou, V., I. Leinonen, and I. Kyriazakis. 2014a. Modelling phosphorus intake, digestion, retention and excretion in growing and finishing pigs: Model description. Animal. 8: 1612-1621.

Symeou, V., I. Leinonen, and I. Kyriazakis. 2014b. Modelling phosphorus intake, digestion, retention and excretion in growing and finishing pigs: Model evaluation. Animal. 8: 1622-1631.

Symeou, V., S. A. Edwards, and I. Kyriazakis. 2012. Modelling digestibility of dietary phosphorus in growing and finishing pigs. J. Anim. Sci. 90: 59-61.

## Conference and abstracts

Symeou, V. 2013. The relationship between phosphorus and protein mass. DSM® monogastric conference, 8-9th October 2013, Dublin, Ireland.

Symeou, V., I. Leinonen, and I. Kyriazakis. 2013. Development of a model to predict phosphorus digestion, retention and excretion in growing and finishing pigs. Proc. Brit. Soc. Anim. Sci. 16-17th April 2013, Nottingham, England. Theatre Presentation.

Symeou, V., I. Leinonen, and I. Kyriazakis. 2013. Evaluating a model that predicts phosphorus digestion, retention and excretion in growing and finishing pigs. Proc. Brit. Soc. Anim. Sci. 16-17th April 2013, Nottingham, England. Theatre Presentation.

Symeou, V., I. Leinonen, and I. Kyriazakis. 2013. Modelling phosphorous intake, digestibility, retention and excretion in pigs. Int. Symp.Modelling Pig and Poult. Prod. São Paulo, Brazil.

Symeou, V., I. Leinonen, S. A. Edwards, and I. Kyriazakis. 2012. Prediction of digestible phosphorus requirements for different pig genotypes. Proc. XII Int. Symp. Digest. Phys. Pigs. Keystone, CO, USA. Poster presentation.

Symeou, V., I. Leinonen, S. A. Edwards, and I. Kyriazakis. 2012. Modelling the digestibility of dietary phosphorus: Model logic and concepts.Proc. XII Int. Symp. Digest. Phys. Pigs. Keystone, CO, USA. Theatre presentation.

Symeou, V., I. Leinonen, S. A. Edwards, and I. Kyriazakis. 2012. Developed a methodology to estimate the phosphorus requirements for different pig genotypes. Proc. Brit. Soc. Anim. Sci. 24-25th April 2012. Theatre Presentation.

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# Chapter 1. Introduction

## 1.1 Phosphorus in pig nutrition and the challenge of modelling its metabolism

Phosphorus (**P**) is a nutrient with many important roles in pig nutrition. It is important for bone mineralization, since 80% of the inorganic fraction of bone consists of calcium phosphate (Frandson et al., 2009). P is also the backbone of RNA and DNA (Anderson et al., 2006) and is involved in nearly every cell function via the energy transfer molecules AMP, ADP and ATP (Bauman, 2004). These vital body functions of P, in conjunction with uncertainty regarding the exact P requirements of the growing and finishing pig and estimation of P digestibility, have lead pig nutritionists to oversupply dietary P (Whittemore et al., 2003) in order to ensure that all animals within a group meet their requirement. A review commissioned by the British Pig Executive (Kyriazakis, 2008) suggested that the UK the pig industry was using P levels in the feeds of all pig classes which were above the recommended Nutrient Requirement Standards (BSAS, 2003). However, the limited global reserves of mineral phosphates (Dourmad and Jondreville, 2007) now cause P to be the third most expensive dietary nutrient after protein (**Pr**) and carbohydrates (energy). The high cost of P is due to the low digestibility of plant dietary P, which makes up the majority of P in the feed, and results in the need to supplement expensive, non-renewable inorganic P to meet the digestible P requirements (Kornegay and Qian, 1996).

The low digestibility of plant P contributes to high P excretion, causing risk of water pollution in the form of eutrophication (Kornegay and Qian, 1996) with recognized detrimental effects on the quality of fresh and salt water bodies (Dourmad and Jondreville, 2007). Eutrophication of surface water results in anaerobic biogenesis of odour compounds (Forsberg et al., 2005) and greenhouse gas emission (Naqvi et al. 2000). It has been estimated that pig production contributes around 14% of the total diffuse P load from livestock to UK waters (White and Hammond, 2006). This eutrophication risk was one of the main reasons for the creation of governmental policies and measures to regulate the disposal of pig manure in the European Union. To protect water quality, manure is now applied based on nitrogen (**N**) requirement of

plants. 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. P-based limits are now enforced in several US states, such as Michigan, Maryland, Virginia and Florida, with several other states and EU countries considering a switch to P-based limits (Bannink et al., 2010). In order to reduce P excreted by growing and finishing pigs, which produce the majority of P excreted in pig meat production, the dietary supply of P needs to be better adjusted to the pig requirements and strategies to improve P digestion and retention need to be developed and implemented.

Mathematical models can be used to reduce the cost of production, as well as the P excretion to the environment, without jeopardizing the pigs' growth rate. They integrate our knowledge on the forms of P in the feed, the biological processes taking place in the gastrointestinal tract for the digestion of P (**digP**) and absorption into the bloodstream, and the pathway of P retained in the body from the bloodstream. Therefore mathematical models can be valuable tools for estimating pig individual requirements for digP in a population, and digP derived from feeds in each unique farm production scenario, and thus can have an important role in providing information that can be used in the decision-making process to enhance the efficiency of the feeding system (Tedeschi et al., 2004). Modelling also increases the effectiveness of experiments and enhances the progress in understanding nutrition (France and Kebreab, 2008) because it identifies physiological functions that are capable of empirical or mechanistic representation and guides research towards optimising the empirical representation.

As a background to the development of a model of P utilisation in pigs described in this chapter we will provide a general background to: (1) metabolism of P; (2) specification of dietary P requirements; (3) the role of phytases; (4) the role of other dietary components in P digestion; and (5) strategies to reduce P excretion.

## 1.2 Metabolism of P in the pig

Whereas uptake of digestible P from the lumen of the small intestine into the blood-stream increases linearly with increasing dietary P concentration (Lopes et al., 2009), digestible P retained in the body has a linear plateau relationship (Ekpe et al., 2002) and is a function of the digestible P requirements. If the digestible P (**digP**) supplied is more than that required to sustain maximal growth, any excess is stored in bone (Campbell, 1965; Vipperman et al., 1974; McGlone, 2000; NRC, 2012). If digestible P supplied exceeds the requirements for maximum bone mineralisation, then there is hyperphosphatemia (Baker, 2011) and the thyroid responds by secreting calcitonin (CT) which down-regulates parathyroid hormone (PTH) (Jongbloed, 1987). The lowered levels of PTH act on the proximal tubules of the kidneys to increase P excretion, mainly through the urinary tract (Jondreville and Dourmad 2005; Koeppen and Stanton, 2009; Suttle, 2010; González-Vega and Stein, 2014). This P excreted is in a soluble form (Kirchmann and Pettersson, 1995) and represents the highest potential risk for losses by runoff in agricultural fields (Maguire et al., 2005).

If, however, the digestible P supplied is less than the requirement to sustain maximal growth, the pig experiences conditions of hypophosphatemia. A lowered P level in the blood triggers the thyroid to increase secretion of PTH (Baker, 2011). PTH causes the osteocytes to release P from bone mass (Baker, 2011). This demineralization process will increase P in the blood, thus helping to sustain maximal Pr deposition at the expense of bone development and the P:Pr ratio in the body decreases (Martínez-Ramírez et al., 2008; Columbus et al., 2010), since between 60 and 80% of total P in the body is stored in the bone tissue (Crenshaw, 2001). If P is deficient in the feed for a prolonged period of time, a medical condition known as rickets will develop (Pond et al., 2005) and deterioration in the animal's condition may be observed.

It also has to be noted that the P metabolism is closely related to calcium (Ca) intake and vitamin D, as they are required for absorption and bone deposition (Koch and Mahan, 1985). Pigs fed a Ca deficient diet were able to absorb more P from the intestine into the bloodstream, because less insoluble and indigestible Ca-P complexes were formed (Selle et al., 2009). Despite more P being absorbed into the bloodstream, if there is insufficient dietary digestible Ca (**digCa**) and vitamin D, then a significant proportion of digP is excreted through the urinary tract. The majority of digestible P within the

body, 70% to 80%, is fixed together with 96% to 99% of Ca to form hydroxyapative, which is the main constituent of bone (Suttle, 2010). It is vital for the formation of hydroxyapative to have a 2:1 ratio between digCa and digP (Létourneau-Montminy et al., 2012). A suggested ratio of digestible Ca to digestible P is between 1.55:1 and 1.70:1, because 20% to 30% of P is found in soft tissues. Nevertheless, the lack of knowledge on Ca maintenance requirements and efficiency of utilisation, as well as our inability to accurately predict the dietary Ca digestibility (González-Vega et al., 2014), requires these recommended Ca:P ratios to be used with caution. A low Ca concentration in the blood will stimulate the production of calcitriol or 1,25-*dihydroxyvitamin D*3 (**1,25(OH)2D3**), a metabolic form of vitamin D that aids in increasing the active absorption of dietary Ca from the lumen of the small intestine. Most feedstuffs contain little or no vitamin D so it must be provided through supplementation (Crenshaw, 2001). Vitamin D needs to be supplemented through the diet (Jongbloed, 1987) because the majority of pig production systems are indoors, where they have limited access to direct sunlight that aids in the production of 1,25(OH)2D3.

### 1.3 Specification of dietary P requirements

Historically, diets for pigs have been formulated on the basis of total P concentration, although it is recognized that P in feed ingredients is not completely digested and retained, and there are differences in the digestibility of P in different feed sources (Sauvant et al., 2004). Since the most recent publication of the American Nutrient Requirements of Swine (NRC, 2012), there has been a widely accepted method for estimating the digestibility of P in different feed ingredients for pigs. The apparent total tract digestibility (**ATTD**) is now the major system for assessing the P value of feedstuffs for pigs, even though it is progressively being replaced by a new system called standardized total tract digestibility (**STTD**) (NRC, 2012) that, unlike ATTD takes into account the endogenous P losses. A major advantage of the STTD method is that values of individual ingredients are believed to be additive in mixed feeds (Baker, 2011). The use of these systems allows a reduction of the safety margin in comparison with total P specification when formulating feeds for pigs (Jondreville and Dourmad,

2006), since they give a more accurate idea of the amount of digestible P delivered to the pig that is available for retention.

Despite the improvements in precision resulting from the use of ATTD and STTD compared to bio-available P (NRC, 1998) or total P feed contents, the system for estimation of requirements still needs to be improved to take into account the other dietary and pig intrinsic factors that may affect the P digestibility. According to this approach there is no unique value of digP content for a feed ingredient, as this would depend on feed composition. This clearly presents a challenge to the industry, who wishes to have estimates of such a value. The dietary factors affecting the P digestibility are: 1) the activity of exogenous and endogenous phytase enzymes; 2) dietary Ca concentration; 3) dietary fibre level; and 4) the interaction of P with other nutrients such as amino acids and micro-mineral cations.

A factorial method is generally used to estimate the P requirements of pigs, based on measurement of the retention of P in the body, obligatory P losses and P digestibility (Guéguen and Pérez, 1981). Total requirement is calculated as the sum of requirements for maintenance and growth taking into account their efficiency of P utilisation for both functions. The equations used for the factorial determination of P requirements for maintenance and growth of pigs are usually a function of BW, as for example in the model of Jondreville and Dourmad (2006). Jongbloed (1987) and Rodehutscord et al. (1998) estimated maintenance P requirements to be 7 mg/kg BW, as inevitable endogenous P losses determined by feeding P-free feeds. More recently, Jondreville and Dourmad (2005) using more improved, leaner pig genotypes, established maintenance P requirements to be 10 mg/kg BW. The disadvantage of the BW expression is that it takes into account fat, which is a variable chemical component in the body (Wellock et al., 2003). Since lipid is an inert component in the body, which does not contain any phosphorus, it has no maintenance requirements. A pig which has a higher body fat content will therefore give rise to an overestimated maintenance P requirement compared to the actual need. Expressing the digestible P requirements per kg BW means that different genotypes will have different requirements at the same BW because of differences in body composition. Even the same genotype at different time scales will have different requirements, e.g. Landrace used in the 1990s will have lower digestible

P requirements compared to today's Landrace pigs because of the intensive selection for body fat reduction over this period. This clearly demonstrates the pitfalls of using BW as a descriptor of requirements (Emmans and Kyriazakis, 1997). Emmans and Kyriazakis (2001) therefore suggested that there would be advantages to be had if digestible P requirements were to be expressed as a function of body Pr.

A more recent modelling approach is the calculation of total endogenous P excretion (**TEPE**) rather than the inevitable endogenous P losses described previously. Shen et al. (2002), Ajakaiye et al. (2003), Petersen and Stein (2006), Pettey et al. (2006), and NRC (2012) calculated the TEPE using a mathematical regression technique. Pigs were fed graded levels of P in the feed and the recovery of P at the faeces/distal ileum was measured. The recovery of P at zero P intake was estimated via a mathematical extrapolation, representing the TEPE (Schulin-Zeuthen et al., 2007). The main advantage of calculating TEPE is the development of a more mechanistic approach, because of the traceability of the endogenous P losses that are digested and re-absorbed, since the inevitable endogenous P losses are only a fraction of the observed total endogenous P secreted in the gut (Nyachoti et al., 1997). TEPE has been expressed as a function of dry matter intake (**DMI**) because most endogenous P excretion originates from the digestive juices, thus feed intake pre-determines the release of the digestive juices to aid digestion. The main problem with expressing TEPE as a function of DMI is that, in modelling DMI ideally should not be used as an input when predicting the P requirements. In modelling the pig growth, DMI is a predicted output of the model, rather than an input previously estimated from experiments. Differences in housing, genotype, feed bulkiness, energy and Pr content all impacts on the DMI (Wellock, et al., 2003) and there is a degree of uncertainty associated with it.

Models that simulate the body P retention have been based on quadratic equations (Jongbloed et al. 1999, 2003; Jondreville and Dourmad 2006; GfE 2008; NRC, 2012). Even though the quadratic equation gives the best fit in comparison to other equations, it is biologically limiting. Apart from anything else, once P retention reaches its maximum, it would remain constant and would not decrease as suggested by the quadratic equation. Jongbloed et al. (1999, 2003), Jondreville and Dourmad (2006) and GfE (2008) calculated the body P retention according to BW or empty BW (**EBW**),

while the NRC (2012) made the maximum P retention rate in the body a function of N retention. There is also a general consensus that the P requirement to maximise bone mineralization and to sustain maximal growth is different (Cromwell et al., 1970; Jondreville and Dourmad, 2006; Van Milgen et al. 2008; NRC, 2012). As far as we are aware, only NRC (2012) estimated that 85% of the level of digestible P intake is needed to reach the maximum performance of the animal, in comparison with the 100% needed to maximize bone mineralisation. Nevertheless, the criteria that NRC (2012) used for deriving the above estimate were not clearly explained. In addition, logic dictates that the bones can provide the body with P for only a limited period of time, depending on the initial bone condition when the offered feed is first limiting in P.

Finally, the effect of BW on the requirement for dietary P concentration (g/kg feed) is well documented and it declines as the pig grows (Suttle, 2010), while the effect that BW has on P digestibility is unknown (Baker, 2011). Jongbloed's (1987) review showed a decreasing P digestibility as body weight increased, while the meta-analysis of Letourneau-Montminy et al. (2012) suggested that pigs of higher body weight had higher P digestibility. Any new models for calculation of P requirements should therefore take account the above considerations.

### 1.4 The role of phytase enzymes

It is well documented that phytase enzyme supplementation can result in major improvements in P digestibility. Phytase enzymes dephosphorylate non-digestible phytate P (**oP**) present as salts of phytic acid (Adeola and Sands, 2003; Beaulieu et al., 2007) into easily digestible phosphate form (**NPP**). Phytate and NPP exist in different ratios and have different concentrations, depending on the feed ingredient. Phytate content as a percentage of total P content varies greatly between cereals; with wheat having one of the highest, while oats and rye have one of the lowest values (Viveros et al., 2000; Sauvant et al., 2004; NRC, 2012). Most cereal by-products have a higher total P and oP content than cereal seeds. The cereal by-products, such as wheat bran, are usually composed of aleurone that is plentiful in oP (Viveros et al., 2000) because this is the place that plants store most of their P content. The percentage of the oP as a

percentage of total P content is lowest for legume seeds. Unlike cereals, in legume seeds oP is distributed throughout the entire protein complex of the seed (Swick and Ivey, 1991). Animal-based ingredients generally have more P than plant feedstuffs. Fish meal has approximately three times more P (31g/kg) compared to rapeseed (11.4g/kg) which is one of the highest P content plant ingredients (Sauvant et al., 2004). In addition, animal-based products do not contain oP and their P content is completely comprised of phosphate. Feed tables of INRA (Sauvant et al., 2004) and NRC (2012) contain all this information for use when formulating diets and simulating feeding scenarios to increase the efficiency of P utilization, therefore decreasing the P excretion to the environment.

In cereal grains, grain by-products and oilseed meals, about 60-75% of the P is in the form of oP, and therefore a significant increase in P digestion can be observed with the supplementation of phytase enzymes (Nelson et al., 1968; Sauvant et al., 2004; NRC, 2012). As much as 60% of oP in the feed can be dephosphorylated into easily digestible phosphate (Kies et al., 2006).There are exogenous plant phytase and microbial phytase enzymes, which are dietary inputs, and also phytase enzymes of endogenous origin, which are found in the small intestine and in the microflora of the large intestine.

Two types of phytases have been described, based on the position of the phosphate group they hydrolyze first (Tamim et al. 2004). The 3- and 6-phytases initiate dephosphorylation at the 3 and 6 position on the oP molecule, respectively (Kies et al., 2001). The action of these 2 phytases differs by how many phosphates they are able to remove from the myo-inositol hexaphosphoric acid molecule, the location sequence of phosphate removal (Woodzinski and Ullah, 1996), and by their activities at different pH levels (Selle et al., 2000). The most widely used exogenous phytase enzymes for supplementation are from fungal *Aspergillus niger* and microbial *Escherichia coli*, a 3-phytase (Kies et al., 2001), as well as from *Peniophora lycii*, belonging to the 6-phytase group (Lassen et al. 2001). Despite the numerous studies investigating the effect of exogenous phytase supplementation in P utilization, there are few publications that document oP dephosphorylation (Olukosi et al., 2013). Only Létourneau-Montminy et al. (2011) modelled this relationship using Michaelis-Menten kinetics for the P dephosphorylation into phosphate by *A. niger* phytase. As far as we are aware, no

author has so far attempted to simulate the effect of other exogenous phytase, e.g. *E. coli*, to dephosphorylate oP into phosphate.

These commercial exogenous phytase supplements seem to be susceptible to thermal treatments and proteases (Simon and Igbasan, 2002), as well as the low pH that characterises the stomach and the duodenum where most of the microbial phytase activity takes place. Bacterial phytases are more thermostable, protease resistant and have a lower acidic pH for optimum activity in comparison to fungal phytases, and this makes them more attractive in feed formulation (Bohn et al., 2007; Olukosi, 2012). New strains of these microbial phytases have been produced that are thermophilic, protease resistant and have pH optima that are within the pH range of the stomach (Quan et al., 2004). Nevertheless, experiments with these 2$^{nd}$ generation products to quantify their impact in P digestion are still somewhat limited.

Most plant phytases are 6-phytase and, like supplemented phytase, are susceptible to denaturation when exposed to high temperatures. They have a lower affinity to oP in comparison to microbial phytase (Lei and Porres, 2003). Rapp et al. (2001) showed that microbial phytase (3-phytase) is more resistant to denaturation in the stomach than plant phytase. The activity of plant phytase decreases very rapidly when the pH is lowered, while the activity of microbial phytase is still around 60% at pH 2 (Eeckhout and De Paepe 1992). These factors explain, at least in part, why plant phytase is 1.4 to 2.5 times less effective in vivo than microbial phytase (Eeckhout and De Paepe, 1992; Weremko et al., 1997; Zimmermann et al., 2002; Jondreville and Dourmad, 2005). Therefore, the importance of plant phytase activity has in the past been underestimated, but studies such as those of Poulsen et al. (2007) and Blaabjerg et al. (2010; 2012) demonstrated their importance in P digestibility. Blaabjerg et al. (2012) attempted to model the effect of oP dephosphorylation for barley and wheat at different soaking times using a generalised Michaelis Menten equation. The Michaelis Menten function permits the fractional rate of oP degradation to decrease continually with time.

The susceptibility of plant phytase is an important reason for the variability in P digestibility resulting from the ingredients' exposure to manufacturing processes, such

as heat-treatment for pellet production and soaking used in liquid feeding systems. Konietzny and Greinet (2002) found that most plant phytase was denatured within minutes above 70$^{o}$C when pelleting took place (Jondreville and Dourmad, 2005). Liquid feeding provides possibilities for improvement of the digestibility of oP, because mixing of feed and water initiates oP degradation before feeding (Lyberg et al., 2005, 2006; Blaabjerg et al., 2010). Soaking of the feed ingredient provides a medium in which endogenous plant phytase can dephosphorylate oP (Blaabjerg et al., 2010). A manufacturing process that is not related to reliance on plant phytase, but directly dephosphorylates the oP, is the production of distillers dried grain with solubles (DDGS). Pedersen et al. (2007) found a greater P digestibility when using DDGS compared to the original grain because some of the bonds that bind P to the oP complex in the grain were hydrolysed during the fermentation process in the ethanol plants.

In contrast to Suttle (2010), who stated that pigs do not synthesize the phytase enzyme required for hydrolysis of oP, the studies of Birge and Avioli (1981) and Maenz and Classen (1998) found that pigs have effective endogenous phytase in the intestinal mucosa to dephosphorylate part of the oP into phosphate (Adeola and Cowieson, 2011). This endogenous small intestine phytase is unique, as it displays an optimal pH in the neutral to alkaline range (Lei et al., 2007). Nevertheless, these endogenous phytase enzymes seem to be adversely affected by the dietary Ca level (Plumstead et al., 2008). Only the model developed by Létourneau-Montminy et al. (2011) took into account the effect of endogenous small intestine phytase on oP dephosphorylation, setting it at a constant 20% apparent oP digestibility. This clearly creates challenges and room for improvement in modelling, as endogenous phytases are affected by dietary factors.

Like phytase in the small intestine, phytase in the large intestine is also affected by dietary Ca (Sandberg et al., 1993), but phytase activity in the large intestine does not increase the P digestion because only small amounts of P are absorbed at this location (Liu et al., 2000; Veum, 2010). The endogenous microbial phytase from the microflora of the large intestine is much more potent in dephosphorylation (Sandberg et al., 1993) and it increases the soluble portion of P excretion. Soluble P is a more significant contributor to eutrophication, rather than the relatively inert insoluble P in the form of

oP and its complexes (Maguire et al., 2005). No author has ever modelled the effect of total dietary Ca on oP dephosphorylation by endogenous phytase enzymes.

### 1.5 The role of calcium in P digestion

It is well accepted that Ca is a major determinant of the extent of P digestion (Selle et al., 2011). The effect Ca on P is mainly through oP, because Luttrell (1993) has shown that oP has 11 times greater affinity to Ca than phosphate. Létourneau-Montminy et al. (2011) also simulated the formation of phosphate-Ca complexes. The negative charges of the oP molecule positively chelate charged multi-valent cations, such as zinc, magnesium and Ca to form stable oP-cation complexes (Kim et al. 2002). Due to the high dietary concentration of Ca in comparison to other cations, the majority of oP-cation complexes are formed as oP-Ca complexes. When phytase enzymes are supplemented into the feed, there is the liberation of digestible Ca; according to Létourneau-Montminy et al. (2012), 500 FTU *A. niger* will deliver 0.64g digestible Ca. The dephosphorylation of oP by phytase enzymes cause a lower dietary concentration of oP for Ca to bind with, therefore more Ca is available for digestion.

An increasing Ca : P ratio in the feed lowers P digestion, resulting in reduced growth and bone calcification and decreased performance (Liu et al., 1998). However, the Ca:P ratio is not important for animal performance if P is supplied in excess in the feed (Vipperman et al., 1974; Mahan, 1982; Hall et al., 1991; Eeckhout et al., 1995). Dietary Ca can have both a direct and indirect impact on phytase activity along the gastrointestinal tract. There is little tangible evidence that Ca directly inhibits exogenous phytase activity (Selle et al., 2009). Ca supplemented in the feed in the form of limestone has a high acid-binding capacity, which may raise the pH of the gastric phase (Eeckhout and De Paepe, 1992). If this causes the pH of the stomach to rise above the pivotal threshold of pH 5, then there is formation of an insoluble Ca-oP complex (Evans and Pierce, 1981; Grynspan and Cheryan, 1983; Oberleas and Chan, 1997) which is resistant to phytase dephosphorylate of oP. Létourneau-Montminy et al. (2011) attempted to simulate the variation of pH in the stomach and its effect on the dephosphorylation of microbial phytase enzymes based on *in vivo* studies.

### 1.6 The role of other dietary components in P digestion

In pigs, dietary fibre is composed of fermentable and non-fermentable fibre. These influence the digesta transit time and are the main substrates for bacteria in the gastrointestinal tract (Metzler et al., 2006). Dietary fibre can lower the pH in digesta of the large intestine as a result of increased volatile fatty acid production, due to fibre fermentation (Metzler and Mosenthin, 2008). The less alkaline condition in the large intestine increases the dephosphorylation of oP and, because the phosphate produced is not digested (Liu et al., 2000; Fan et al. 2001; Shen 2006), it will increase the potential for an environmental problem. Soluble fermentable fibre makes digesta viscous (Cherbut et al., 1990), resulting in a reduced mixing of dietary components with endogenous digestive enzymes, eventually causing a lower P digestibility (Metzler and Mosenthin, 2008). Feeding non-fermentable fibre increases the transit time and improves gut morphology by increasing villi length and stimulating mucosal enzyme activity (Hedemann et al., 2006). There is also growing evidence of growth promotion of beneficial bacteria, such as lactobacilli and bifidobacteria, therefore maybe increasing P digestibility, by increasing the endogenous phytase enzymes. Nevertheless, both fermentable and non-fermentable fibre sources increase intestinal epithelial cell proliferation rate, therefore increasing the maintenance P requirements (Metzler and Mosenthin, 2008), and special attention should be paid to the hypothesis that higher microbial P utilization may reduce the P availability for the host animal (Metzler and Mosenthin, 2008). Taking into account the complexity of the impact of dietary fibre on P digestion and the scarcity of data, no author has yet attempted to simulate this parameter, even though it merits attention.

The microminerals may also play a role in the availability of P (NRC, 1998; Kornegay, 2001; Baker, 2011). The presence of high levels of Fe, Al, and Mg may adversely affect the absorption of P by the pig. High levels of these minerals may form complexes with P resulting in reduced P digestibility, making it important to maintain the proper balance among minerals in the feed (NRC, 1998). Due to the lack of data, the impact that micro-minerals have in the P digestion was not considered any further in this thesis.

Finally, oP may interact with other food components in the digestive tract, such as amino acids and starch, and hence have adverse effects on pig growth rate. However, from a recent review of Selle et al. (2012) it was concluded that there is conflicting and inconclusive information on this topic and thus it is very difficult to quantify using mechanistic approaches due to a lack of data. There is a general consensus that the negatively charged oP molecule forms insoluble binary oP-Pr complexes, through the formation of salt-like linkage with the basic amino acid residues, but this takes place only at a pH less than their isometric point (Cosgrove, 1966; Cheryan, 1980; Anderson, 1985). Once the protein isometric points are exceeded, usually in higher pH environments of the small intestine, binary complexes dissociate, but they still may not be as readily digested in the small intestine due to structural changes induced by aggregation with oP (Selle et al., 2012). Some ingredients, having a high isoelectric point, such as wheat, persist with the binary Pr-oP complexes even through the small intestine (Ravindran et al., 1999). There are strong indications that phytase enzymes can increase the Pr utilisation through the dephosphorylation of oP, and therefore fewer oP-Pr complexes are formed (Selle et al., 2000).

### 1.7 Modelling approaches to P digestion, utilisation and excretion

As described in the previous sections, one of the most advanced models of P digestibility was created by Létourneau-Montminy et al. (2011). The core of the model is based on a compartmental structure which distinguishes three parts where the following successive processes take place: 1) P solubilisation and phytic hydrolysis; 2) P absorption; and 3) formation of P-Ca complexes. The model divided the digestive tract into sections that are involved in the P and Ca digestion: 1) the gastric area where there is the partial solubilisation of the oP ingested, and its eventual dephosphorylation by exogenous phytase occurs; 2) the proximal small intestine where a portion of phosphate is absorbed; and 3) the distal small intestine where certain compounds become insoluble because of an increase in the pH. The model of Létourneau-Montminy et al. (2011) is a dynamic model as transit time also modulates the extent of oP dephosphorylation. The model predicted ATTD in pigs, while as described previously, it is more accurate to incorporate the endogenous P losses and predict the STTD (NRC, 2012).

A few models have simulated the P metabolism in pigs (Fernández, 1995; Vitti et al., 2000; NRC, 2012). Fernández (1995) presented a model based on isotope dilution to study P movement between the following compartments: 1) gut lumen; 2) plasma; and 3) bone. The same isotopic method was used by Vitti et al. (2000), but they added soft tissue and gastro-intestinal tract compartments. In developing the model, the conservation of mass principle was applied to each pool to generate differential equations that account for P exchanges between pools.

The most complete and recent model on P digestion, utilisation and excretion comes from the updated NRC (2012). One of the changes from NRC (1998) to NRC (2012) was that values for relative bioavailability of P were no longer used. Instead, values for SSTD of P in all feed ingredients were also provided in the feed composition tables. The theoretical basis for this model is the calculation of P requirements based on N retention, because a straight line relationship between body contents of N and P has been observed (Stein, 2012). It is also concluded that the requirement for P to maximize body weight gain and feed efficiency is only 85% of the P needed to maximize bone mineralisation.

Although some of the above models are dynamic, i.e. they account for changes in the above processes over time, most of them do not take into account the variation between pigs in requirement, digestion and utilisation; none of the existing models take into account the uncertainty in P content that may arise from feed ingredients and processing. The NRC (2012) model is in principle stochastic, although no suggestions are made towards this. This is because this model recognises the different P requirements between the sexes and genotypes.

### 1.8 Stochastic approaches to model P digestion, utilisation and excretion

Reducing P excretion involves close matching of dietary P supply to animal requirement in farm practice. This either requires optimisation at a population level, or availability of technology to differentially feed individual animals. Most mathematical pig growth models simulate the growth of only the ‘average’ individual within the herd (population) and assume that this is a good representation of the population (Ferguson, 2013). Nevertheless, several studies (Knap, 2000; Pomar et al., 2003; Wellock et al.,

2004; Patience and Beaulieu 2006; Brossard et al., 2009) have shown that the overall mean of the population responses can differ significantly from the average individual response due to the variation in genetic (growth potential), as well as other sources of variation in population distribution: health, feed, physical and social environment. The more individuals vary within a population (or the greater the weight variation), the more inappropriate it is to use the average individual response as a means of predicting the optimal population response. For example, predicting nutrient requirements for a population based on the single deterministic response will introduce a bias against individuals with a higher nutrient requirement.

The first challenge resolved by the scientific community in order to develop a stochastic model was the quantification of variation in the genetic traits of pigs. The majority of authors (Knap, 2000; Pomar et al., 2003; Wellock et al., 2004) used the following genetic traits to simulate an individual pig: (1) protein at maturity (**$Pr_m$**); (2) lipid to protein ratio at maturity (**$LPr_m$**); and (3) scaled maturing rate (**B***). They used the mean values and their standard deviation (**SD**) to characterise the population, from which the values that characterize each animal are drawn before each simulation run and are able to be maintained for multiple simulation runs (Wellock et al., 2004). The more recent approach used by Brossard et al. (2009) and Vautier et al., (2013) for simulating an individual pig is through the following genetic traits: 1) average daily gain between 65 days of age and 110 kg of BW; 2) BW at 65 days of age; 3) scaled maturing rate; 4) expected daily feed intake at 50 kg BW; 5) shape parameter of the FI. The challenge with using the more recent approach has been raised in the previous section, as FI should be a model output and not a model input.

The social stressors are another model parameter that has been considered to affect population requirements by Wellock et al. (2004). Variation in the consequences of social "stressors" exists between genotypes, where it has been suggested that leaner, more modern genotype pigs tend to be less able to cope. It is expected that, within a population or group, the social environment (i.e., position within the social hierarchy) also affects an individual's ability to cope. In this model, it was assumed that the larger, more dominant individuals are better able to cope when exposed to social stressors. Consequently, within a population, the effects of social "stressors" are correlated with

body weight around the genotype mean. In this way, Wellock et al. (2004) were able to investigate the effect of group size and space allowance on population performance based on the above assumptions. More recently Sandberg et al. (2006) considered the effects of the infectious environment on the requirements of a pig population. Like Wellock et al. (2004), this approach raises the issue of how to describe the pig in terms of its ability to cope with the various stressors, including its exposure to pathogens. This thesis will not consider explicitly the effects of the thermal, social and infectious environments any further, but will assume that no limitations are imposed on the pig from these environmental components.

Although the scientific community had developed models that can take into account genetic and environmental variation, it has not considered variation in feed composition and its consequences. This has been shown to have a significant effect on the population growth performance (Kim et al., 2002; Groesbeck et al., 2007; Weis et al., 2012). Feed ingredients may vary substantially in nutrient composition, due to growing conditions, hybrid or variety differences, planting and harvest dates, storage and feed out conditions (Kim et al., 2002). In addition uncertainty in feed composition may arise from the feed manufacturing activities, such as mixing and processing (Traylor et al., 1994; Groesbeck et al., 2007; Pedersen et al., 2007). One of the main objectives of this thesis is to develop a methodology to allow us to investigate: (1) how uncertainty about feed composition (arising from variability in ingredient nutrient content and mixing efficiency) would affect the outputs of a nutrient utilization simulation model, and (2) how such uncertainty would interact with the uncertainty that arises from the genetic traits of individual pigs within a population.

### 1.9 Strategies to reduce P excretion

Application of precision farming is gaining momentum, as it is an agricultural management concept that recognises the existence of in-field variability (Pomar and Pomar, 2012). It is based on the fact that animals within a group differ from each other in terms of growth potential and their response to environmental constrains, and therefore nutrient requirements (Pomar et al., 2003; Wellock et al., 2004; Brossard et al., 2009). Precision feeding involves the use of feeding techniques that allow the right

amount of feed with the right composition to be provided at the right time to each pig in the herd (Pomar and Pomar, 2012), see **Figure 1.1**.

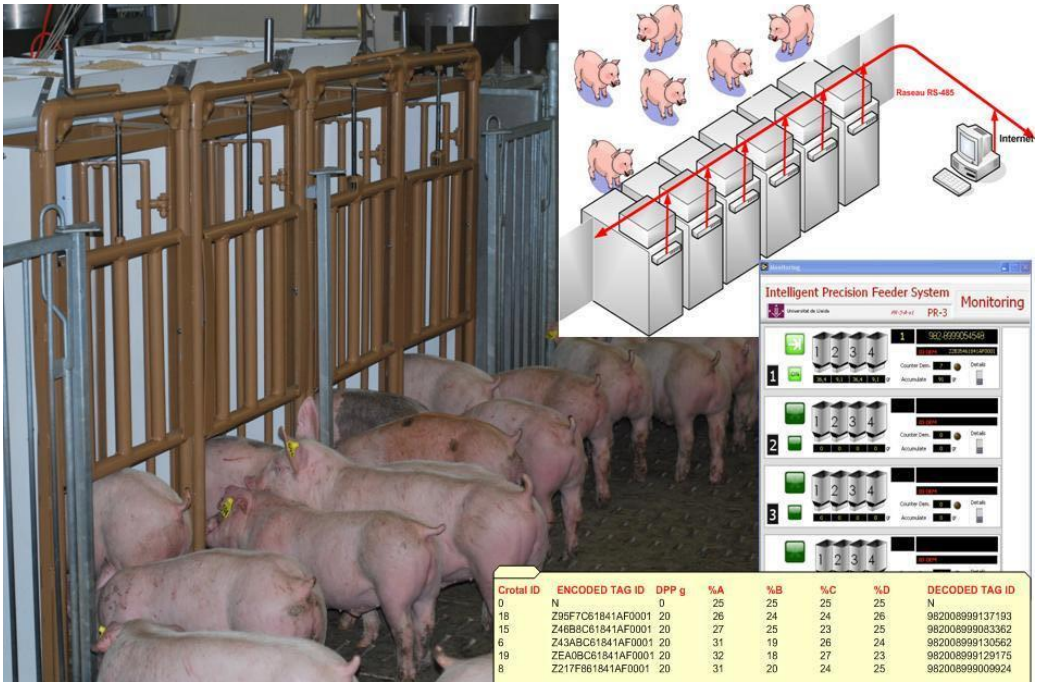


**Figure 1.1** The automatic and intelligent precision feeder for individual tailored feeding (Pomar et al., 2009).

The most promising and practical feeding strategies focus on two main principles: minimizing input and maximizing the efficiency of utilization (Simpson and de Lange, 2012). There are a number of management options available to reduce the P excretion in growing and finishing pig systems. Some of these strategies are simple and can significantly affect the P excretion, since they involve supplementation of phytase enzymes, and provision of the correct Ca:digestible P ratio through avoidance of excess supplementation of limestone. This is necessary to prevent the adverse impact of excess Ca on P digestion, whilst also providing sufficient Ca (1.55-1.70:1 ratio with P is considered to be optimal) so that bone formation can take place as hydroxyapative (Veum, 2010).

Management strategies that can potentially reduce P excretion without jeopardizing pig growth include phase feeding and sorting according to BW. Sorting involves the

regrouping of pigs to give pen groups with reduced body weight variation and hence more uniform P requirement. Phase feeding involves the design of an increased number of different feeds given sequentially as the pigs grow and change P requirements. These strategies, alone or in combination, allow better matching of P supply to current population requirement and reduce over-feeding of digestible P which will simply be excreted (Simpson and de Lange, 2012). Although both these strategies have been experimentally investigated (Lenis, 1989; Coppoolse et al., 1990; Henry and Dourmad, 1993; Pomar et al., 2009; 2011; O'Quinn et al., 2000; Schinckel et al., 2005; 2007), a consistent quantification of their effects on pig performance and P excretion is lacking from the literature.

The above was one of the main objectives of this thesis. Through development of a stochastic model we were able to quantify the consequences of these feeding strategies on: (1) the cumulative P excretion as total, soluble and insoluble P (kg); (2) the population performance (mean and CV) in terms of BW gain (kg/d), Pr and P retained (g/d) and food conversion ratio; (3) the percentage of the population that have their digP requirements met throughout the BW period 30 to 120 kg; and (4) the percentage of the population that are supplied with less than 85% of their requirements at any stage of their growth and might be expected to show reduced performance.

### 1.10 Overview of the thesis

The overall aim of the work described in this thesis is to develop and validate a modelling tool which can assist the pig industry to improve P utilisation and thus reduce pollution potential. Specific objectives to achieve this aim were: (1) To develop a dynamic, mechanistic model of P utilisation in the pig using the most up to date scientific information and concepts; (2) To extend this model to operate at a population level, by the incorporation of genetic variation; (3) To use the model to evaluate the impact of possible farm strategies to reduce P excretion; and (4) To develop a full stochastic model by the incorporation of variation in the feed.

Based on the literature outlined above, Chapter 2 of this thesis brought together the various factors that affect P digestibility in a quantified manner, to develop a dynamic, mechanistic model for P intake, digestion, retention and excretion for the average growing and finishing pig. This allowed the estimation of P digestion, retention and excretion in relation to diet composition and pig genotype; the approach also enabled estimates of P requirements for different pig genotypes. In Chapter 3, a sensitivity analysis was conducted to investigate how variations in the key parameters affect model predictions. In addition, Chapter 3 described the model evaluation to quantify the accuracy of the model against independent experimental studies. Therefore the model deficiencies were identified, as well as the areas where future effort should be directed.

However, the practical application of such a model is somewhat limited because the concepts apply to a single, average animal (van Milgen et al., 2008). In reality, decisions are made for groups of animals sharing a common feed and therefore Chapters 4 and 5 describe further development of the model to estimate the digestible P requirements of a population of pigs and the fate of dietary P. Chapter 4 focused on the practical aspects of the model by investigating different strategies, namely phase feeding and sorting and their impact in growth performance and P digestion, retention and excretion. Chapter 5 then developed the model into a full stochastic form, by also incorporating the dietary variation and investigated how such dietary variation interacted with the uncertainty that arises from the genetic traits of individual pigs within a population. In addition, Chapter 5 investigated the impact on P retention, growth performance and P excretion, when feeding the population with a feed having high inherent variability, in comparison to conventional dietary variability. The final chapter (Chapter 6) is a general discussion of the major findings of this research, and conclusions.

# Chapter 2. Modelling phosphorus intake, digestion, retention and excretion in growing and finishing pigs: model description

## 2.1 Abstract

Low phosphorus (**P**) digestibility combined with intensive pig production can increase P diffuse pollution and environmental load. The aim of this paper was to develop a deterministic, dynamic model able to represent P digestion, retention and ultimately excretion in growing and finishing pigs of different genotypes, offered access to diets of different composition. The model represented the limited ability of pig endogenous phytase activity to dephosphorylate phytate as a linear function of dietary calcium (**Ca**). Phytate dephosphorylation in the stomach by exogenous microbial phytase enzymes was expressed by a first order kinetics relationship. The absorption of non-phytate P from the lumen of the small intestine into the blood stream was set at 0.8 and the dephosphorylated phytate from the large intestine was assumed to be indigestible. The net efficiency of using digested P was set at 0.94 and assumed to be independent of body weight, and constant across genotype and sex. P requirements for both maintenance and growth were made simple functions of body protein mass, and hence functions of animal genotype. Undigested P was assumed to be excreted in the feaces in both soluble and insoluble forms. If digestible P exceeded the requirements for P then the excess digestible P was excreted through the urinary flow; thus the model represented both forms of P excretion (soluble and insoluble) into the environment. Using a UK industry standard diet, model behavior was investigated for its predictions of P digestibility, retention and excretion under different levels of inclusion of microbial phytase and dietary Ca, and different non-phytate P:phytate ratios in the diet, thus covering a broad space of potential diet compositions. Model predictions were consistent with our understanding of P digestion, metabolism and excretion. Uncertainties associated with the underlying assumptions of the model were identified. Their consequences on model predictions, as well as the model evaluation are assessed in the next chapter.

## 2.2 Introduction

Phosphorus is an important mineral for both the metabolism and skeletal development of the growing pig (NRC, 2012). In pig diets, P is the third most expensive nutrient required, after carbohydrates (energy) and protein. The high cost of P is due to the low digestibility of plant dietary P, which results in the need to supplement with expensive, non-renewable inorganic P to meet the digestible P requirements (**dPreq**) of the animals (Selle et al., 2011).

The low digestibility of P also contributes to high P excretion, causing water pollution, in the form of eutrophication (Selle et al., 2011). The low digestibility of dietary P is because the majority of plant P is in the form of phytate P (**oP**), which needs to be dephosphorylated by phytase enzymes and liberate 6 molecules of phosphates that are available for absorption (**NPP**). Phytate dephosphorylation is mainly affected by: (1) exogenous phytase enzymes, which are composed of plant and microbial phytases; (2) endogenous phytase enzymes, found in the small, as well as in the large intestine; (3) pH of the gastrointestinal system; (4) digesta flow rate; and (5) dietary Ca concentration (Létourneau-Montminy et al., 2011).

*In silico* experimentation through mathematical modelling offers a feasible alternative to experimentation to investigate the consequences of management treatments that aim to maximize P digestion and retention, whilst minimizing P excretion. Using our current understanding of P digestibility and the principles of retention, a mechanistic model may be developed that could incorporate all factors affecting retention and excretion, in order to decrease P excretion and the use of expensive inorganic P, leading to both environmental and economic benefits. The main aims of this paper were to: (1) develop a dynamic, deterministic model that would translate total dietary P (**tP**) into dP; (2) simulate dP retention; (3) estimate P excretion in terms of both insoluble P and the more environmentally-hazardous soluble P by growing and finishing pigs. In addition, the developed model was tested for the responses of different pig genotypes offered foods of different compositions, in terms of tP content.

## 2.3 Material and methods

### *2.3.1 A general overview of the model*

A deterministic, dynamic model was developed; it consists of four modules; referred to as: (1) Digestion; (2) Food intake; (3) Retention and; (4) Excretion. Compartmental models are often used to describe the fate of different nutrients along the gastrointestinal tract (Dias et al., 2010). The compartmental model of **Figure 2.1** is the most appropriate, as it generates the lowest residual sum of squares based on the study of Létourneau et al. (2011). A more complex model, i.e. considering the effect of soluble and insoluble fiber and its effect in the transit time of the digesta might be inappropriate. Occam's razor would lead us to choose the current model over more complex models, because with a fewer estimable parameters there is a lower residual sum of squares. It is acknowledged that using compartmental models we assume that all the material that is added to or removed from the system is described in the model and that the digesta in the compartment is homogeneous.

The overall inputs to, and outputs from the model are listed in **Table 2.1**. A list of abbreviations used in the model description is shown in **Table 2.2**. A schematic description of the digestion module of the model is shown in **Figure 2.1**. Its first step was the consideration of tP (g/kg diet) as oP (g/kg diet) and phsphates (g/kg diet). The P digested in the 'digestion' part of the model was expressed in g/kg diet. There was the need to estimate food intake (**FI**) in g/day in order to estimate the actual P digested, retained and excreted in g/day (see below). The P requirements, dPreq were calculated as a function of maintenance and maximum P retention, taking into account the inefficiency of P utilisation for growth. Any dP (g/day) supplied in excess of the requirements was excreted through the urine as soluble P.

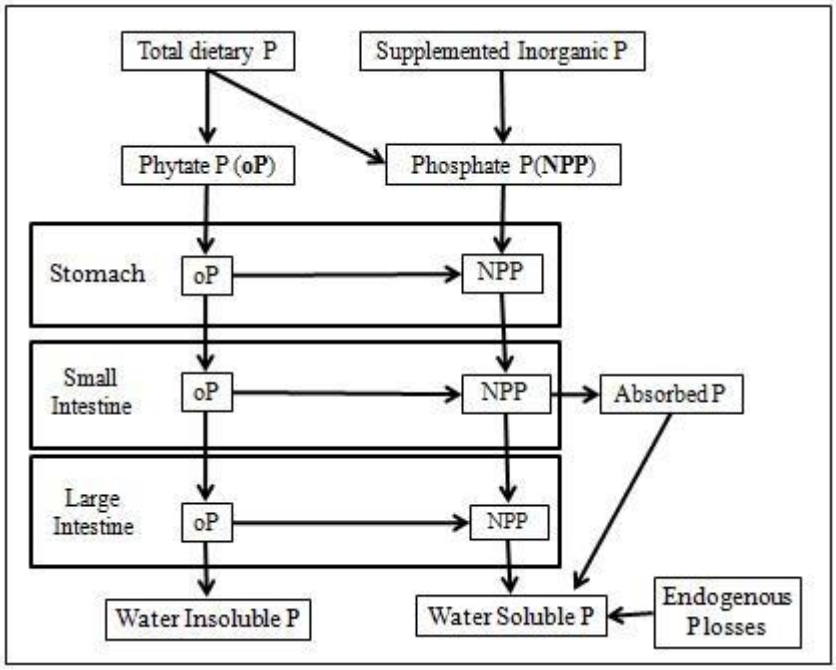


**Figure 2.1** Schematic description of the model of phosphorus (**P**) intake, digestion, retention and excretion. Food resources contain phytate (**oP**) and phosphates P (**NPP**). Phytate is dephosphorylated in the stomach by exogenous microbial and plant phytase. In the small and large intestine the oP dephosphorylation takes place by endogenous phytase. The remaining oP is excreted as insoluble P, while the endogenous P excreted and phosphate that is neither digested nor utilised are excreted as soluble P.

**Table 2.1** Model inputs and main model outputs.

| Inputs | Outputs |
|---|---|
| Start body weight (kg) | Intake (kg/day) |
| End body weight (kg) | Average daily food intake ($FI_a$) |
| Diet formulation | Average daily desired FI ($Fi_d$) |
| Food composition | Phosphorus digested ( g/day) |
| Digestible energy content (DEC, MJ/kg) | Phosphorus retained (g/day) |
| Crude protein content (CPC, g/kg) | Phosphorus excreted (g/day) |
| Water-holding capacity (kg water / kg DM) | Urine phosphorus excretion |
| Supplementing microbial phytase (FTU/kg) | Faecal phosphorus excretion |
| Feed processing | Soluble phosphorus excretion |
| Pig description | Insoluble phosphorus excretion |
| Growth rate parameter (B/day) | Final body phosphorus composition (kg) |
| Mature protein mass ($Pr_m$, kg) | |
| Mature lipid mass ($L_m$, kg) | |

**Table 2.2** Abbreviations of names for entities used in the model

| Abbreviation | Description |
|---|---|
| $A.niger_{dephos}$ | Dephosphorylation of phytate by *A.niger* phytase (kg/kg oP) |
| B | Gompertz coefficient of growth ($day^{-1}$) |
| BW | Body weight (kg) |
| BW0 | Initial body weight (kg) |
| Ca | Calcium |
| dP | Digestible P |
| $dP_{input}$ | P absorbed from the lumen available for retention (g/day) |
| dPreq. | Digestible P requirements (g/day) |
| $E.\ coli_{dephos}$ | Dephosphorylation of phytate by *E. coli* phytase (kg/kg oP) |
| EBW | Empty body weight (kg) |
| $e_{growth}$ | Efficiency of P utilization for growth |
| $e_{maint}$ | Efficiency of P utilization for maintenance |
| $EP_{losses}$ | Minimum endogenous P losses associated with maintenance losses (g/day) |
| FI | Feed intake (kg/day) |
| FTU | Phytase activity |
| $inefP_{ret}$ | P losses because of the inefficiency of digestible P utilization (g/day) |
| $k_2$ | Isometric coefficient relating P retention to body protein retention |
| $Kmax._{A.niger}$ | Maximum phytate dephosphorylation by *A. niger* phytase (g/g) |
| $Kmax._{E.coli}$ | Maximum phytate dephosphorylation by *E. coli* phytase (g/g) |
| $Kmax._{LI}$ | Maximum phytate dephosphorylation by endogenous large intestine phytase (g/g) |

| | |
|---|---|
| KmaxPlant | Maximum phytate dephosphorylation by plant phytase (g/g) |
| $Kmax._{SI}$ | Maximum phytate dephosphorylation by endogenous small intestine phytase (g/g) |
| $LI_{dephos}$ | Dephosphorylation of phytate by endogenous large intestine phytase (kg/kg oP) |
| $L_m$ | Body lipid at somatic maturity (kg) |
| $MaxP_{ret.}$ | Maximum digestible P retention (g/day) |
| NPP | Easily Digestible phosphate |
| $NPP_{indig.}$ | Phosphorus losses due to the inefficiency of P absorption into the bloodstream (g/day) |
| oP | Phytate phosphorus (g/day) |
| *p* | Constant coefficient for maintenance phosphorus (g/day) |
| P | Phosphorus |
| $Plant_{dephos}$ | Dephosphorylation of phytate by plant phytase (kg/kg oP) |
| $P_{maint}$ | Digestible P requirements for maintenance (g/day) |
| Pr | Body protein mass (kg) |
| $Pr_m$ | Body protein mass at somatic maturity (kg) |
| PrR | Daily protein retained (g/day) |
| R*A.niger* | Rate parameter for phytate dephosphorylation by microbial *A. niger* phytase |
| R*E.coli* | Rate parameter for phytate dephosphorylation by microbial *E.coli* phytase |
| $R_{LI}$ | Rate parameter foe phytate dephosphorylation by large intestine phytase |
| RPlant | Rate parameter for phytate dephosphorylation by plant phytase |
| $R_{SI}$ | Rate parameter for phytate dephosphorylation by small intestine phytase |
| $SI_{dephos}$ | Dephosphorylation of phytate by endogenous small intestine phytase |

| | |
|---|---|
| $sP_{losses}$ | Soluble P losses (g/day) |
| tP | Total phosphorus |

### *2.3.2 Representation of P digestion*

The P in plant feedstuffs consists of non-digestible oP and easily digestible phosphates in different concentrations. Inorganic P and animal based feedstuffs do not contain oP, but may have different P digestibilities (Sauvant et al., 2004). Data for establishing the oP and phosphate content of feedstuffs included in any diet were primarily taken from the INRA feed tables produced by Sauvant et al. (2004).

In the model, the process of P digestion begins in the stomach, provided that no fermentation of the feed ingredients took place prior to feeding (Blaabjerg et al., 2012). In the stomach, supplemented microbial and/or plant phytase dephosphorylates oP into phosphates. The plant phytase activity of each feed ingredient can be found in the comprehensive feed tables of Sauvant et al. (2004), which were used to derive diet plant phytase activity (FTU/kg). The model takes into account that if the feed ingredients were exposed to temperatures of more than $80^{o}$ C (Nair et al., 1991), mainly by steam-pelleting, a 50% reduction in plant and microbial phytase activity would take place (Jongbloed and Kemme, 1990). The decrease in phytase activity occurs because phytase is prone to denaturation after exposure to high temperatures. Nevertheless, modern stains of supplemented microbial enzymes are coated and retain their dephosphorylating activity until temperatures of $80^{o}$C (Rasmussen, 2010).

Diurnal variation in pH, such as before and after feeding (Kidder and Manners, 1978), has the potential to influence the activity of phytase enzymes, the solubility of oP (Selle and Ravindran, 2008) and the formation of insoluble and indigestible Ca-oP complexes (Selle and Ravindran, 2008). However, in the model, it was assumed that possible fluctuation in pH does not affect P digestibility and consequently no oP-Ca complexes were assumed to be formed in the stomach. A first-order kinetics equation to represent the oP dephosphorylation by plant phytase was used:

$$oP_{dephos} = \mathbf{Kmax} \cdot (1 - e^{-R \cdot FTU}) \quad \textbf{(2.1)}$$

where, $oP_{dephos}$ is the amount of oP dephosphorylated per unit of oP (kg/kg oP), *Kmax* is the maximum ratio of oP dephosphorylation (i.e. the total amount of "reactive" phytate), FTU is the phytase activity, and *R* is the rate parameter.

A first-order kinetics equation (**Equation 2.2**) to represent the oP dephosphorylation by plant phytase was fitted to the data by Sauvant et al. (2004). The equation shows a response where initially with increasing levels of phytase there is a nearly linear increase of rate of dephosphorylation, while at very high phytase levels the curve has reached an asymptote, indicating that all the "reactive" phytate has been dephosphorylated within the given time. Sauvant et al. (2004) provided two values for apparent faecal P digestibility for feed ingredients with a significant endogenous phytase activity: wheat, wheat bran, rye, barley and triticale. The first value corresponds to the feed ingredient digestibility when phytase has been denatured, e.g. by exposing the feed ingredient to extreme heating. The second value, which is higher, corresponds to the same feed material when it was processed in a way that does not affect phytase activity, cool milling for instance. The difference between the two digestibility values was assumed to be the contribution of plant phytase activity by oP dephosphorylation. With a passive transport of 0.8 phosphate absorption from the lumen of the small intestine into the blood-stream (Gunther, 1978; Jongbloed, 1987), the oP dephosphorylation by plant phytase can then be estimated, see **Figure 2.2**.

$$\boldsymbol{Plant_{dephos}} = \mathbf{KmaxPlant} \cdot (\mathbf{1} - \boldsymbol{e^{-Rplant \cdot FTU}}) \qquad \textbf{(2.2)}$$

where, $Plant_{dephos}$ is the amount of oP dephosphorylated per unit of oP (kg/kg oP), *KmaxPlant* is the maximum ratio of oP dephosphorylation (i.e. the total amount of "reactive" phytate), with a value of 0.337, *FTU* is the phytase activity, defined as the amount of enzyme that liberates 1µmol of inorganic P in 1 minute from 5.1 mmol solution of sodium oP at 37$^{o}$C at pH 5.5, and *Rplant* is the rate of the response of oP dephosphorylation against FTU (kg/kg oP), with a value of 0.00217. The coefficient of determination ($R^2$) of the fitted relationship to the mean data of Sauvant et al, (2004) was 0.854 and the root mean square error (RMSE) was 0.0391 kg/kg oP.

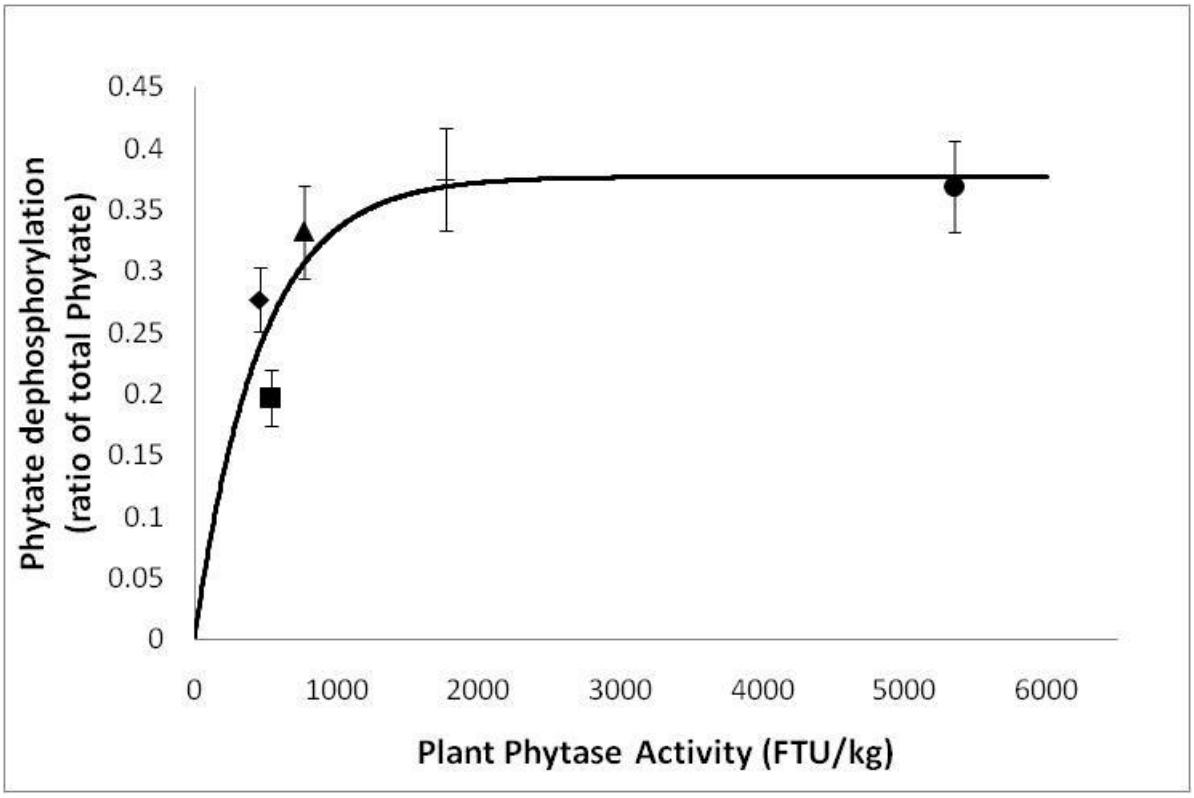


**Figure 2.2** The relationship between plant phytase activity and proportion of phytate dephosphorylation, for: (■) barley; (♦) wheat; (▲) triticale; (+) wheat bran; and (●) rye. The fitted relationship was $0.337 \times (1 - \exp(-0.00217 \times \text{FTU})$; $R^2 = 0.854$; RMSE= 0.0391 kg/kg oP. The values were derived from mean data and SE of Sauvant et al. (2004).

Microbial phytases are currently used widely in grower and finisher pig diets. There are different types of microbial phytase, but this paper quantified the effects of two main categories of phytase enzymes: 3- and 6- phytases, derived from *Aspergillus niger* and *Escherichia coli* (Adeola et al., 2006). The *E. coli* phytase has a single pH optimum range (2.5 to 3.5), which is different from the two pH optimals of 2.5 and 5.5 for the fungal 3-phytase from A. *niger* (Rodriguez et al., 1999).

Quantifying the effect of microbial phytase enzymes on the oP dephosphorylation required studies that used graded levels of microbial phytase up to very high FTU, "super-dosing" (Cowieson et al., 2011), in order to identify the rate (R*E.coli* and R*A.niger*) and maximum ratio ($K_{max}$.A.niger and $K_{max}$.E.coli) of dephosphorylation, by

fitting an exponential equation. The studies used for this purpose should entail feed ingredients, which contained minute, preferably no plant phytase enzymes, so as to solely investigate the effect of supplementation with microbial phytase enzymes. The model considered the dephosphorylation of oP by microbial and plant phytase in the stomach as being additive, in accordance to Zimmermann et al. (2001), provided that oP is not a limiting substrate.

Little research exists, other than the studies of Adeola et al. (2004) and Kies et al. (2006), on the addition of microbial phytase to diets at much higher levels than industry recommended ones (500-1500 FTU/kg), due to marginal returns per unit of supplemental microbial phytase. The *in vitro* study of Adeola et al. (2004) investigated supplementation with *E. coli* phytase, whilst the *in vivo* study of Kies et al. (2006) investigated *A. niger* phytase supplementation; both studies super-dosed the diets with microbial phytase.

Kies et al. (2006) examined the apparent P digestibility, rather than oP dephosphorylation. Expressing the effect of microbial phytase activity in terms of total P digestion fails to take into account the potentially negative effect of dietary Ca and the digestion of dephosphorylated oP separately from the digestion of plant digestible phosphate. The difference of the two digestibility values, with and without microbial phytase is the contribution of microbial phytase activity by oP dephosphorylation. The absorption of phosphate from the lumen of the small intestine into the blood stream was set at 0.8 (Gunther, 1978; Jongbloed, 1987). A non-linear response of supplemental phytase on oP dephosphorylation was observed for both phytases, **Figure 2.3**. Once oP dephosphorylation was calculated, first-order kinetics **equations 2.3 - 2.4** were fitted to the observed results for *A. niger* and *E.coli*.

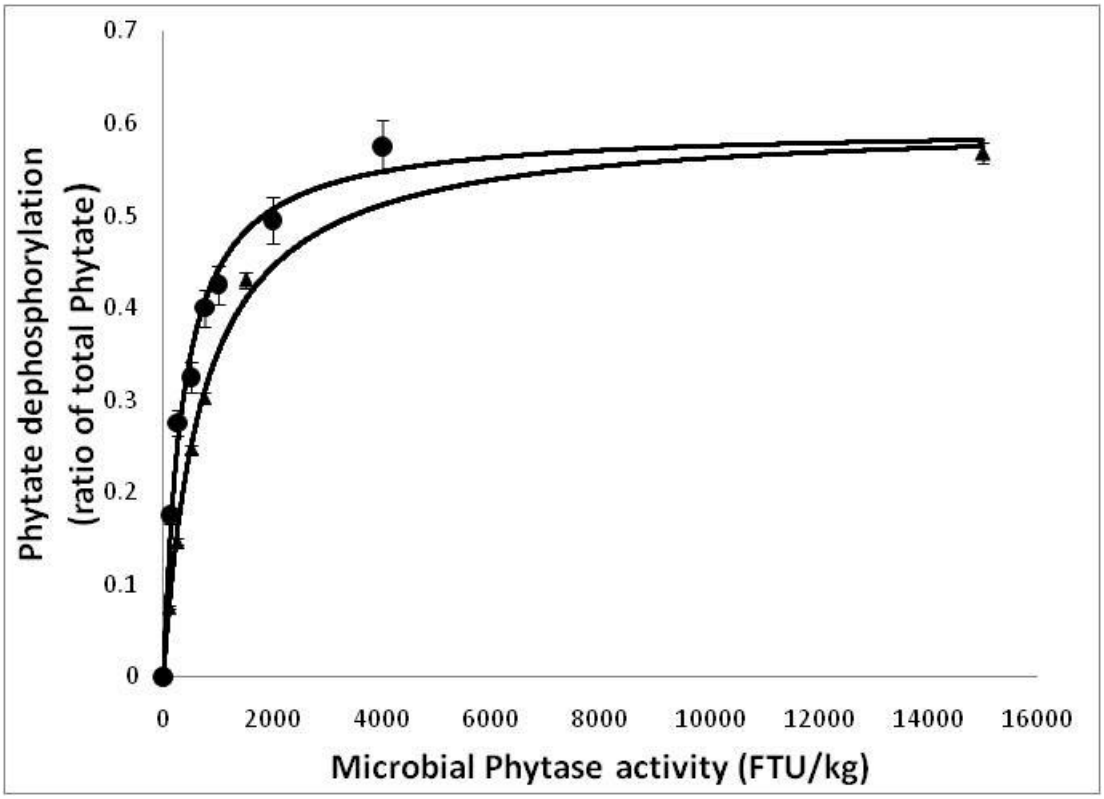


**Figure 2.3** The relationship between *E. coli* (●) and *A. niger* (▲) microbial phytase activity and phytate dephosphorylation. The equations were: 0.532 × (1 – exp(-00187× FTU); $R^2$=0.93; RMSE= 0.0221 kg/kg oP and 0.562 × (1 – exp(-0.00104 × FTU); $R^2$= 0.920; RMSE= 0.0324 kg/kg oP, for *E.coli* and *A. niger*, respectively. The values were derived from the *in vitro* study of Adeola et al. (2004) (●) and the in the *in vivo* study of Kies et al. (2006) (▲) for *E.coli* and *A. niger* phytase activity, respectively.

Kies et al. (2006) examined the apparent P digestibility, rather than oP dephosphorylation. Expressing the effect of microbial phytase activity in terms of total P digestion fails to take into account the potentially negative effect of dietary Ca and the digestion of dephosphorylated oP separately from the digestion of plant phosphate. The difference of the two digestibility values, with and without microbial phytase is the contribution of microbial phytase activity by oP dephosphorylation. The absorption of phosphate from the lumen of the small intestine into the blood stream was set at 0.8 (Gunther, 1978; Jongbloed, 1987). A non-linear response of supplemental phytase on oP dephosphorylation was observed for both phytases, **Figure 2.3**. Once oP dephosphorylation was calculated, first-order kinetics **equations 2.3 - 2.4** were fitted to the observed results for *A. niger* and *E.coli*.

$$A.niger_{dephos} = \text{Kmax}A.niger \cdot (1 - e^{-RA.niger \cdot FTU}) \quad (2.3)$$

$$E.coli_{dephos} = \text{Kmax}E.coli \cdot (1 - e^{-RE.coli \cdot FTU}) \quad (2.4)$$

where, *A. niger*$_{\text{dephos}}$ and *E. coli*$_{\text{dephos}}$ are the amounts of oP dephosphorylated per unit of oP (kg/kg oP) by *A. niger* and *E. coli*, respectively, while Kmax*A.niger* and Kmax*E.coli* are the maximum ratios of oP dephosphorylation (kg/kg oP) for *A. niger* and *E. coli*, respectively, with a value of 0.562 and 0.532, FTU is the phytase activityand R*E.coli* and R*A.niger* are the rates of the response of oP dephosphorylation against FTU (kg/kg oP) with values of 0.00104 and 0.00187 for *A. niger* and *E. coli*, respectively. The $R^2$ of the fitted relationship was 0.920 and 0.932 for *A. niger* and *E.coli*, respectively, while the RMSE was 0.0324 and 0.0221 kg/kg oP, respectively. The fact that the maximum ratios of oP dephosphorylation are between 0.5-0.6 probably reflects the fact that oP becomes a limiting substrate at high level of exogenous phytase inclusion. Another limiting factor for incomplete dephosphorylation is that both 3- and 6-phytases that are available commercially are not capable of liberating the C-2 axial phosphate group from phytate (Cowieson et al., 2013).

The model does not take into account any plant and microbial phytase activity in the small intestine, due to the combination of the low solubility of phytate and the ability of protease enzymes to denature phytase (Zhao et al., 2010). Phytate that is not dephosphorylated into phosphate in the stomach and duodenum rapidly scavenges Ca in the small intestine, due to the alkaline environment (Selle and Ravindran, 2008). These Ca-oP complexes are insoluble and not available for dephosphorylation by the endogenous small intestine phytase, therefore they move into the large intestine and are excreted as insoluble P (see **Figure 2.1**). Consequently, these complexes limit the availability of both Ca and oP. It is assumed that the small intestine phytase activity is fixed and does not change with age, in accordance with Létourneau-Montminy and Narcy (2010).

In the absence of suitable pig data, the study of Plumstead et al. (2008) with broilers was used to quantify the dephosphorylation of oP by endogenous smallintestine phytase enzymes using graded dietary Ca levels (see **Figure 2.4**). It is appreciated that this

assumes that the same principles of P digestion apply across pigs and chickens, despite evidence to the contrary Applegate et al. (2003). The linear equation derived was:

$$SI_{dephos} = Kmax_{SI} - R_{SI} \cdot Ca \quad \textbf{(2.5)}$$

where, $SI_{dephos}$ is the amount of oP dephosphorylated per unit of oP that enters the small intestine (kg/kg oP), $Kmax._{SI}$ is the maximum ratio of oP dephosphorylation, with a value of 0.261, Ca is the dietary Ca in (g/kg); and $R_{SI}$ is the slope of the response of oP dephosphorylation against Ca (kg/kg oP), with a value of 0.0158. The $R^2$ of the fitted relationship was 0.834 and the RMSE was 0.0531 kg/kg oP. **Equation 2.5** suggests a maximum oP digestibility of 26%, which is in agreement with the study of Jongbloed et al. (1992) and the suggestion of Létourneau-Montminy and Narcy (2010) for pigs.

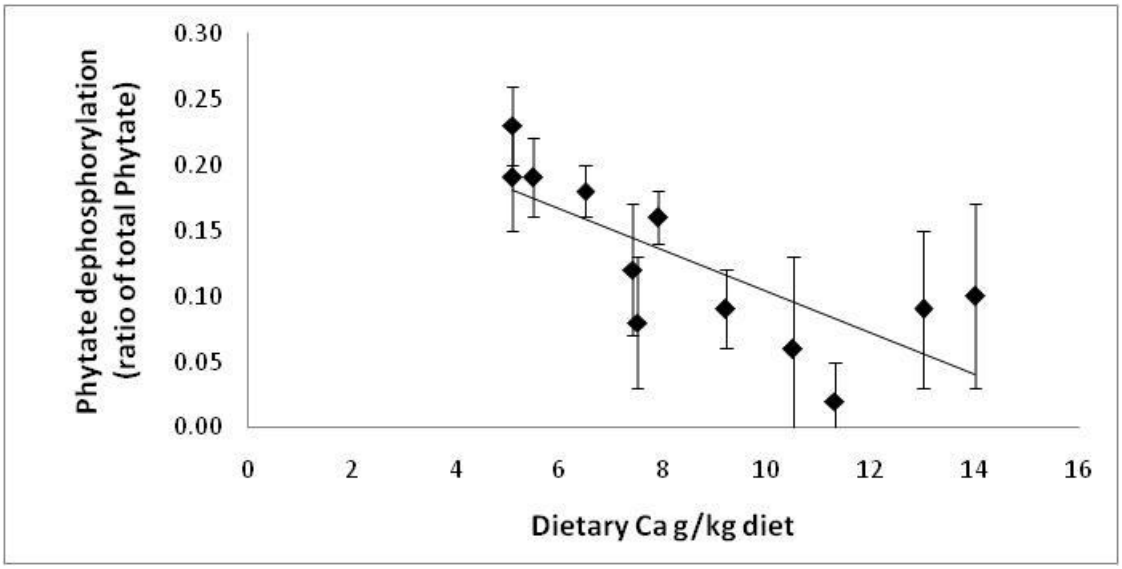


**Figure 2.4** The relationship between dietary Calcium (g/kg) and proportion ofphytate dephosphorylation in the small intestine, based on the in *vivo* ileum cannulated experiment of Plumstead et al. (2008). The line fitted assumes a linear relationship between thetwo variables: 0.261–(0.0158 *(Ca g/kg diet) $R^2$ = 0.834; RMSE= 0.0531 kg/kg oP.

Létourneau-Montminy et al. (2011) suggested that there is the theoretical creation of calcium-phosphate complexes in the small intestine. However, Luttrell et al. (1993) have shown that oP has 11 times greater affinity to Ca than phosphate. For this reason, it was decided not to model the formation of Ca phosphate because in any realistic pig diet there will always be enough oP reaching the small intestine to form oP-Ca complexes over calcium phosphate complexes. Thus the majority of phosphate which

reached the small intestine would be digested into the bloodstream, and could thus overestimate phosphate absorption from a diet high in dietary Ca. The insoluble Ca-oP complexes cannot be dephosphorylated by the large intestine microfloral phytase and are excreted as insoluble P (Selle and Ravindran, 2008). The main problem with quantifying the dephosphorylation of oP in the large intestine is that one cannot be certain of the actual amount of oP available for dephosphorylation. This is because there are no data in the literature that quantify the amount of Ca-oP complexes that take place in the small intestine.

Studies, such as Sandberg et al. (1993) using ileum cannulated pigs, have measured the oP complexes that exit the small and enter the large intestine, but do not distinguish between the inert Ca-oP complex and oP that enters the large intestine. They then measure the amount of oP going out of the large intestine intothe faeces. The linear equation to express the dephosphorylation of the phytatewhich enters the large intestine was expressed as:

$$LI_{dephos} = Kmax_{LI} - R_{LI} \cdot Ca \quad (\mathbf{2.6})$$

where, $LI_{dephos}$ is the amount of oP dephosphorylated per unit of oP that enters the LI (kg/kg oP), $Kmax._{LI}$ is the maximum ratio of oP dephosphorylation with a value of 1.00, Ca is the dietary Ca in g/kg; and $R_{LI}$ is the slope of the response of oP dephosphorylation against Ca (kg/kg oP) with a value of 0.0756. The $R^2$ of the fitted relationship was 0.792 and the RMSE was 0.339 kg/kg oP. The large RMSE could be attributed to the small sampling size.

### *2.3.3 Prediction of food intake*

There was the need to predict FI (kg/day) in order to estimate the actual P digested as g/day. The model does not assume that the pig increases feed intake when P is a limiting nutrient (Emmans and Kyriazakis, 2001), as there is no evidence in the literature that a pig will attempt to eat for a mineral, such as P, when this is the first limiting nutrient in the diet (Pomar et al., 2006; Lopes et al., 2009), but the FI of the pig may be greatly depressed on diets severely deficient in P (Mahan 1982; Lopes et al., 2009). Following

these assumptions, 'desired' FI was calculated by dividing the requirement for a digestible feed resource (MJ or g/day) by the digestible protein or net energy content of the diet (MJ or g/kg diet) (Wellock et al., 2003). The only constrained assumed to operate and stopping the pig from meeting its requirements through FI, was the bulkiness of the feed (Kyriazakis and Emmans, 1995), although it appreciated that other constrains may operate in pig systems (Wellock et al., 2003a, 2004; Sandberg et al., 2007) .

### *2.3.4 Representation of P retention*

For a diet first limiting in P and when digested P intake is below P requirements, all dP is assumed to be retained in the body, after maintenance P requirements have been met. Although it is appreciated that endogenous P losses, which constitute a large proportion of the P maintenance requirements, maybe a function of FI (Pettey et al., 2006), maintenance P requirements were assumed to be independent of FI.

Emmans and Kyriazakis (2001) suggested that there would be advantages if maintenance requirements for any diet resource were to be expressed as a function of body Pr. Following the scaling rules used by Emmans et al. (1986) the maintenance P requirements were proposed to be:

$$\mathbf{P_{maint}} = \boldsymbol{p} \cdot \mathbf{Pr} \cdot \boldsymbol{Pr}_m^{-0.27} \qquad \textbf{(2.7)}$$

where, $P_{maint}$ is maintenance P requirements (g/day), $\boldsymbol{p}$ is a coefficient (g/day) expected to be constant across pig genotypes (Emmans and Kyriazakis, 2001), $Pr$ is the pig actual body protein weight (kg) and $Pr_m$ is its protein content at somatic maturity (kg).The value of $\boldsymbol{p}$ was estimated to be 0.1293 (g/day) from the data of Jongbloed (1987), by making the following assumptions: (i) the relationship between Pr and body weight is allometric (Whittemore et al., 1988) and (ii) the $Pr_m$ of the crossbred Dutch Landrace x Dutch Yorkshire gilts used was 30 kg (Knap, 2000). The advantages of **equation 2.7** over existing estimates of $P_{maint}$ are that it can be applied across pig sizes and genotypes, and account for genetic change. The dP efficiency of utilisation for replenishment of maintenance P requirements ($\mathbf{e_{maint}}$) was equal to unity in accordance to Rodehutscord

et al. (1998) and consistent with the estimate of the efficiency of utilisation of other nutrients for maintenance (Sandberg et al., 2005).

The efficiency of dP utilisation maybe calculated from the slope of the regression of net P retention (g/day) against dP intake (g/day) (Pettey et al., 2006). The net efficiency of utilisation of dP for growth ($\mathbf{e_{growth}}$) may depend on pig size, varying from 0.89-0.97 for growing and finishing pigs respectively (Pettey et al., 2006). Because of lack of information, the value of $e_{growth}$ was assumed to be constant across sexes (NRC, 2012) and constant across pig genotypes at 0.94 in accordance with Rodehutscord et al. (1999); Petersen and Stein (2006); Pettey et al. (2006); NRC (2012).

The rate of whole-body P retention was assumed to relate to whole-body protein (**Pr**) mass. From the experiments of Rymarz et al. (1982); Jongbloed (1987); Hendriks and Moughan (1993); and Mahan and Shields (1998) which estimated the empty body (**EBW**) composition of pigs under non limiting conditions, it was found that the relationship between body P and Pr /kg in the EBW was isometric, (**Figure 2.5):**

$$\mathbf{Max}P_{ret} = k_2 \cdot \mathbf{PrR} \qquad (\mathbf{2.8})$$

where, $MaxP_{ret}$ is the maximum body P retention (g/day) and $k_2$ is the isometric coefficient gainer, with a value of 0.0337 and *PrR* is the protein retention in g/day. The $R^2$ of the fitted relationship was 0.99 and the RMSE was 0.003. NRC (2012) have also put forward a clear and close relationship between whole-body P mass and whole-body N mass, but suggested that this was allometric (quadratic). All other body components (i.e. water, lipid and ash) were also assumed to relate to whole-body protein (**Pr**) mass through allometric, for the first two, or isometric relationships for ash (Wellock et al., 2003).

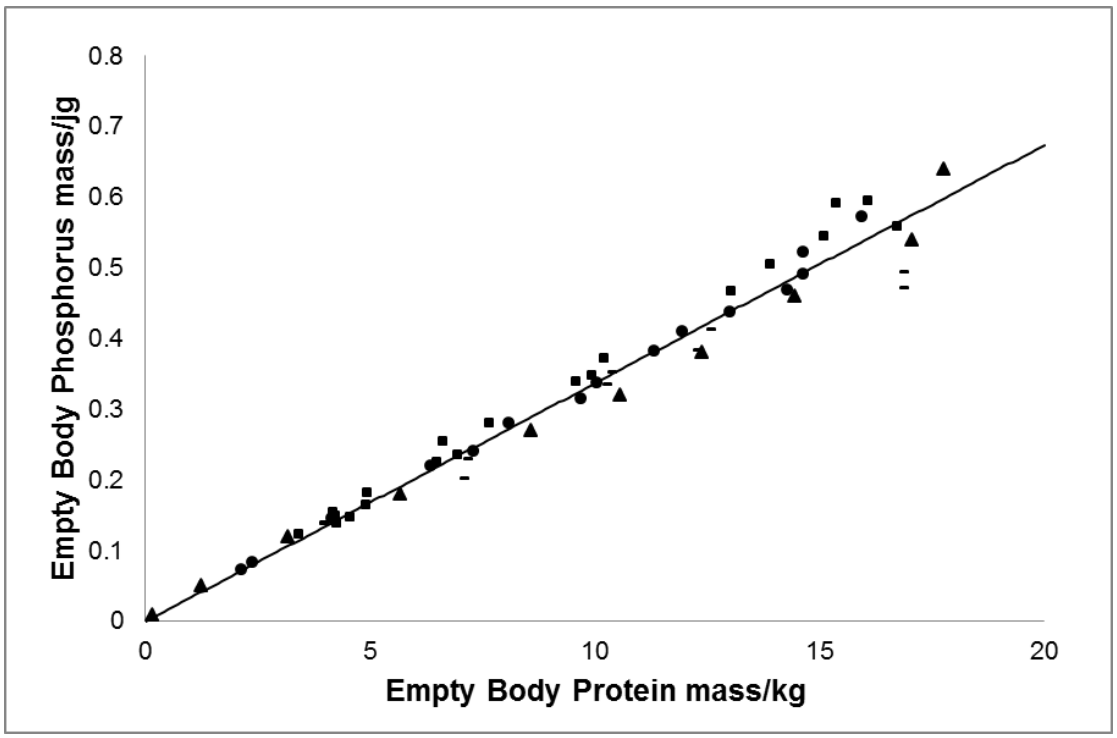


**Figure 2.5** The isometric relationship between protein (kg) and phosphorus (kg) in the empty body of pigs. Data was derived from different experiments: Jongbloed (1987) used gilts (n=22) of a Landrace x Large White breed (■); Rymarz et al. (1982) used gilts (n=65) of a Norwegian Landrace x (Large White x Hampshire) hybrid (●); Hendriks and Moughan (1993) used gilts (n=36) of a Landrace x Large White breed (—); and Mahan and Shields (1998) used gilts and boars (n=81) of a Hampshire x (Large White x Duroc) hybrid (▲)

When the offered diet is first limiting in a nutrient other than P, such as protein, and the pig is fed *ad libitum* then the isometric relationship between the two body components may be assumed to be preserved. However, under certain circumstances, such as when the pig is offered limiting amounts of an imbalanced diet low in Pr but high in P, the P:Pr ratio in the body can increase, indicating that there may be more bone (ash) than protein mass development, and the isometric relationship between P and Pr is disturbed (Kyriazakis and Emmans, 1991). The converse can be the case when the pig is offered limited amounts of a diet low in P but high in protein or amino acids (see **equation 2.9**). In these cases, the P:Pr ratio in the body may decrease as has been shown by Martinez-Ramirez et al. (2008) and Columbus et al. (2010). These relationships can be expressed as follows:

$$P_{ret} = \min\left[(dP_{input} - P_{maint.} - \left[(dP_{input} - P_{maint}) \cdot (1 - e_{growthp})\right], \max P_{ret}\right] \quad (2.9)$$

where, $dP_{input}$ is P absorbed from the lumen to the bloodstream, $P_{maint}$ is dP requirements for maintenance (g/day), $maxP_{ret}$ is the maximum dP retention (g/day), $e_{growth}$ is the efficiency of dP utilisation for growth.

When a pig is given *ad libitum* access to a balanced food and kept under non limiting conditions (in terms of energy and protein), it is expected to meet its requirements and attain maximum growth. The maximum growth of the pig was defined in accordance with Wellock et al. (2003). The individual pig was described by three genetic characteristics: protein weight at maturity (**Pm**, kg), the ratio of lipid to protein at maturity (**Lm/Pm**, kg/kg), and a growth rate parameter (**B**, per day). The initial state of the pig is described by initial body weight (**BW0**, kg) from which the chemical composition of the pig is calculated assuming the pig has its ideal composition set by its genotype. The potential rate of protein retention (**PrR**, kg/d) is determined by pig genotype and current protein weight only.

Given the arguments above, **equation 2.8** is used to determine the potential gains P retention. Therefore the requirements of a pig attaining its maximum growth for P were expressed as:

$$dP_{req} = \frac{P_{maint.}}{e_{maint}} + \frac{MaxP_{ret}}{e_{growth}} \quad (2.10)$$

where, $dP_{req}$ is digestible P requirements (g/day), $P_{maint.}$ is dP requirements for maintenance (g/day), $e_{maint}$is the efficiency of dP utilisation for maintenance, $MaxP_{ret.}$ is the maximum dP retention (g/day), $e_{growth}$ is the efficiency of dP utilisation for growth.

### *2.3.5 Estimation of soluble and insoluble P excretion*

Indigestible P excreted in the faeces is in soluble and insoluble forms. P digested but not utilised, for example when P is supplied above P requirements, is excreted in the urine

and is assumed to be in soluble form (Kirchmann and Petterson, 1995). The latter represents the highest potential risk for losses by runoff in agricultural fields (Maguire et al., 2005). The model was able to distinguish between these two different forms of P excreted.

The insoluble P is only found in the faeces and is composed of oP (mainly in $IP_6$, but also $IP_3$-$IP_5$ forms) and any divalent cation complexes (Selle et al., 2011; Mukhametzyanova et al., 2012). Soluble P excretion originates from urinary P excretion, and contains a fraction of soluble P excreted in the faeces, see **equation 2.11**. The faecal soluble P excretion results from the inefficiency of absorption of phosphate into the blood-stream and originates from dietary and dephosphorylated phosphate as well as endogenous P excretion.

P excreted in the urine comprises of the inefficiency of dP utilisation and any dP which exceeds the dP requirements. The pig does not have any biological process to store excess dP (Ekpe et al., 2002), thus dP intakes which exceed requirements are excreted through the urine as soluble P.

$$\boldsymbol{sP_{losses} = P_{maint} + NPP_{indig} + inefP_{ret} + max\left[\left(dP_{intake} - dP_{req}\right), 0\right]} \quad \textbf{(2.11)}$$

where, $sP_{losses}$ are the soluble P losses (g/day), $P_{maint}$ are the maintenance P losses (g/day), $NPP_{indig}$ are the P losses of the inefficiency of phosphate absorption from the gastrointestinal lumen into the bloodstream being set at 0.2 of the phosphate in the digesta (g/day), $inefP_{ret}$ are the P losses because of the inefficiency of digestible P utilisation being set at 0.1 of all the P that has been absorbed by the lumen of the small intestine into the blood-stream (g/day), $dP_{intake}$ is the digestible P available for retention, and $dP_{req}$ is the digestible P requirements.

### *2.3.6 Running the model*

The model is capable of predictions over both the grower and finisher periods. The model was run over a daily time step using the list of inputs (**Table 2.1**) from a start BW, until a target BW was reached. At the end of each day the gains of each of the four

chemical components (Pr, L, ash, including P and water) achieved were added to the current mass of the four body components to give the new current composition of EBW and hence BW (Wellock et al., 2003).

### *2.3.7 Investigating model behaviour*

The model was used to investigate its predictions over a range of diet and pig genotypes; this is equivalent to assessing model behaviour. The default values used for the model were a certain kind of pig given *ad libitum* access to a standard diet, and the pig was assumed to grow over the period of 30-60 kg BW. The default pig genotype used was characterized by BSAS (2003) as being of 'intermediate growth' with 40 kg $Pr_m$, 48 kg lipid at maturity (**Lm**) and 0.01175 per day growth parameter, which roughly represents the current pig genotypes (LW x L) used in commercial units in the UK. The 'standard' diet used by the UK pig Industry, had the following analysed chemical composition: 9.6 MJ Net energy/kg, 172.5g crude protein/kg, 11.07g lysine/kg, 5.19g tCa /kg and 4.29g tP/kg of feed as fed. The dietary tP consisted of 2.47g oP/kg and 1.82 g phosphate /kg diet. The default diet consisted of wheat - 600 g/kg, barley-110 g/kg, rapeseed meal-80 g/kg, soybean meal- 77g/kg and sunflower meal-50 g/kg fresh diet. The incorporation of wheat made the diet high in plant phytaseat 480 FTU/kg, but the feed was assumed to be pelleted. In addition, the diet was supplemented with an extra 750 FTU *E.coli* microbial phytase according to industry recommendations. The diet was assumed to be abundant in vitamin D. The level of tCa in the diet was slightly lower than BSAS (2003) recommendations and reflected the concerns of the Industry over the recommended level.

Model outputs were produced for the following variations in the default diet to investigate model behaviour: (1) different levels of microbial *E.coli* phytase supplemented to the diet; (2) different total Ca levels in the diet; and (3) different phosphate:oP ratios in the diet. In addition the model was used to investigate the effect of different pig genotypes on the digestible P requirements for both growing and finishing pigs. The outputs of the model predicted were: FI, tP input, digested and retained P, and hence soluble and insoluble P excretion.

Given the curvilinear relationship of oP dephosphorylation with phytase a greater number of lower levels of phytase inclusions were considered, rather than high ones. The NRC (2012) and BSAS (2003) suggest the optimum tCa:digestible P ratio in the pig diet to be 2.6 and 2.8 respectively. In reality however, the optimal tCa:digestible P depends on the actual digestible P in the diet and therefore there is a circularity in this argument. A wide range of dietary tCa was investigated: from 2g/kg diet, which is the dietary Ca contributed from the feed ingredients, to 11g/kg diet when there is the supplementation of Ca carbonate to the diet.

In the past, diets have been formulated on total P basis. However a diet which contains the same tP concentration can result in completely different P digestibility. Phytate and phosphate contents of the diet are the single most important factors to take into account when formulating a diet. A wide range of phosphate:oP ratio were tested in order to find the highest P retention and lowest P excretion for a diet with a 4.29 g/kg diet tP; the lowest ratio tested was 0.4 as conventional diets do not go below this level.

The effect of genotype on model outputs was investigated by using genotype characterised by BSAS (2003) as: 'Commercial growth' with 30 kg $Pr_m$ , 39 kg $L_m$ , 0.011 B; and 'Fast growth' with 50 kg $Pr_m$ , 55 kg $L_m$ , 0.0125 B, in addition to the default genotype of 'Intermediate growth'. Over the BW range considered the three genotypes have an average growth rate of 0.67, 0.97 and 1.25 kg/day respectively.

## 2.4 Results

Outputs for the P requirements of pigs of different genotypes are given in **Figure 2.6**. As expected pigs with higher growth characteristics had higher requirements for digestible P at any stage of their growth. For example, the dP requirement at 80 kg body weight was 5.16 g/day, 6.59 g/day and 8.10 g/day, for the commercial, intermediate and fast growth potential genotypes, respectively.

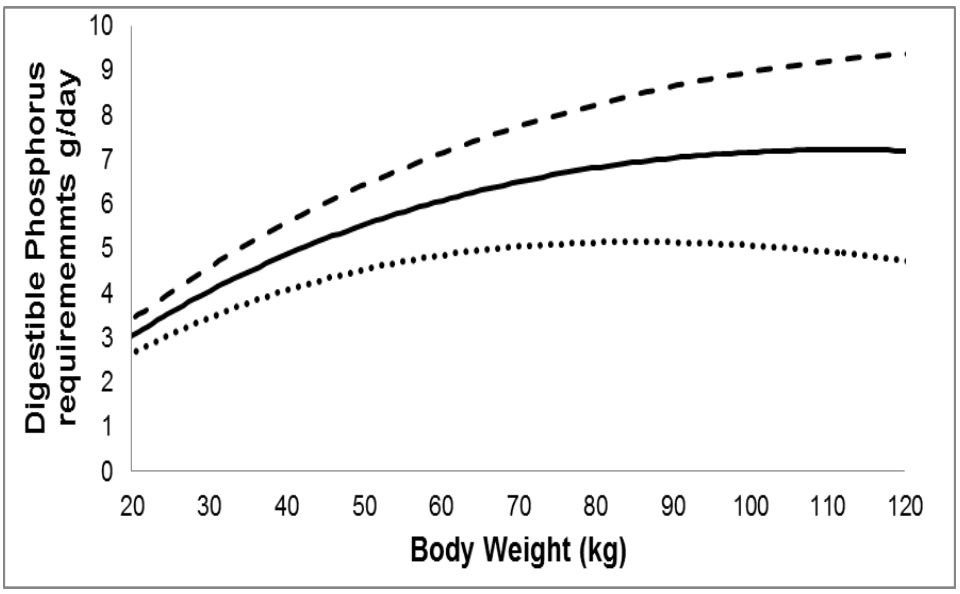


**Figure 2.6** The digestible P requirements of three pig genotypes as defined by BSAS (2003). The 'lean' (– –), 'intermediate' (—) and 'commercial' (···) pig genotypes have: 0.0125, 0.01175, 0.011 growth rate parameter (B, per day) 50, 40, 30 mature protein mass ($Pr_m$, kg) and 1.1, 1.2, 1.3 lipid to protein ratio, respectively.

Increasing the phytase content of the diet increased the P digestibility and retention. For example adding 500 FTU phytase increased the P digestion from about 5 g/day to about 6 g/day (**Figure 2.7**). The response was curvilinear, and P retained reached a constant maximum rate at approximately 1000 FTU, earlier than the maximum P digested rate achieved, at approximately 2500 FTU. Phytate dephosphorylation did not take place beyond supplementation with 2500 FTU phytase. Any additional supplementation of phytase beyond 1000 FTU, resulted in an increase in soluble P excretion, because the dP requirements had been met and the excess dP was excreted through the urinary tract. This implies that the diet used was first limiting in dP, up to the level of inclusion of 1000 FTU *E. coli*. Therefore the optimal level of *E. coli* inclusion for this diet and specific genotype was 1000 FTU.

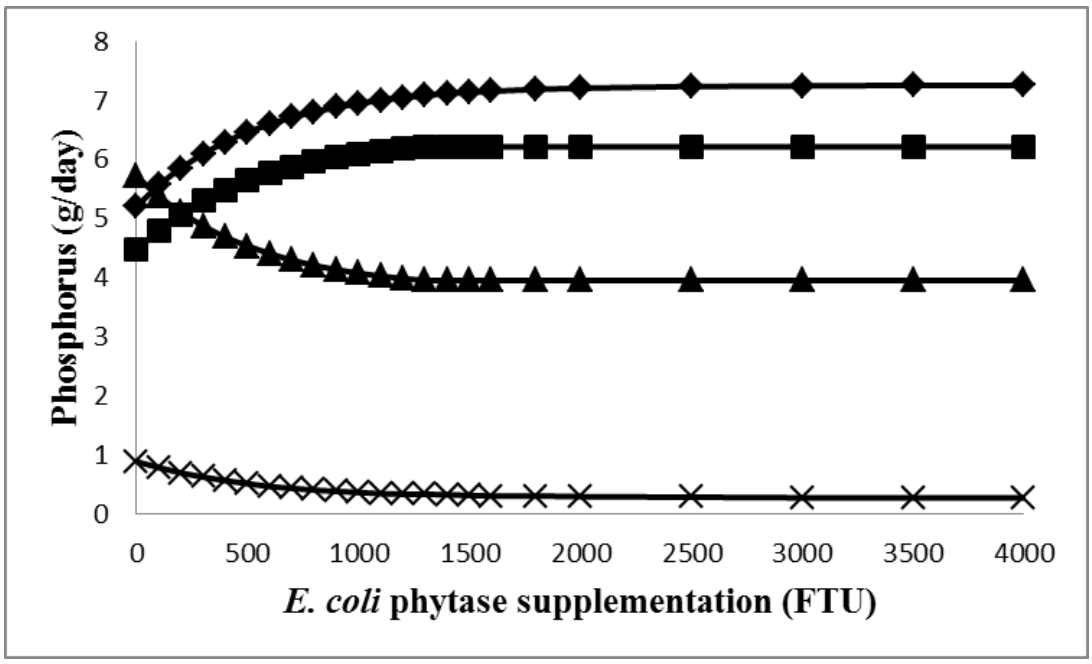


**Figure 2.7**.The effect of microbial *E.coli* phytase supplementation on digestible (♦) and retained (■) phosphorus and hence total (▲) and insoluble P (x) excretion (g/day) for a typical UK commercial diet, containing 5.19 g/kg total Ca and 4.29 g/kg total P, separated into 2.48 phytate and 1.81 phosphate P. The diet also contained a 480 FTU plant phytase. Pigs of an "Intermediate" BSAS (2003) genotype were simulated to grow from 30-60 kg body weight.

Increasing the level of dietary Ca supplementation whilst keeping the rest of the diet composition constant, resulted in a decrease in P digestibility and retention (**Figure 2.8).** There was also an increase in insoluble P excretion, which resulted in a decrease in soluble P excretion. This was the result of the formation of insoluble Ca-oP complexes in the small and large intestine. It is important to note, that even at the very low dietary Ca levels, when the digestibility of P was at its highest level, the dP intake was not enough to meet pig requirements. The maximum dP intake achieved was 6.84 g/day, while dP requirements were 7.08 g/day for this pig genotype. The simulations should be used with caution, as the P digestibility would be even lower than currently predicted at high dietary Ca, as the supplemented Ca will increase the pH of the stomach and create more Ca-oP complexes. The model does not currently account for such a pH effect. Another feature not included in the current version of the model is the possible reduction of P retention under low levels of dietary Ca. This will be discussed in detail below.

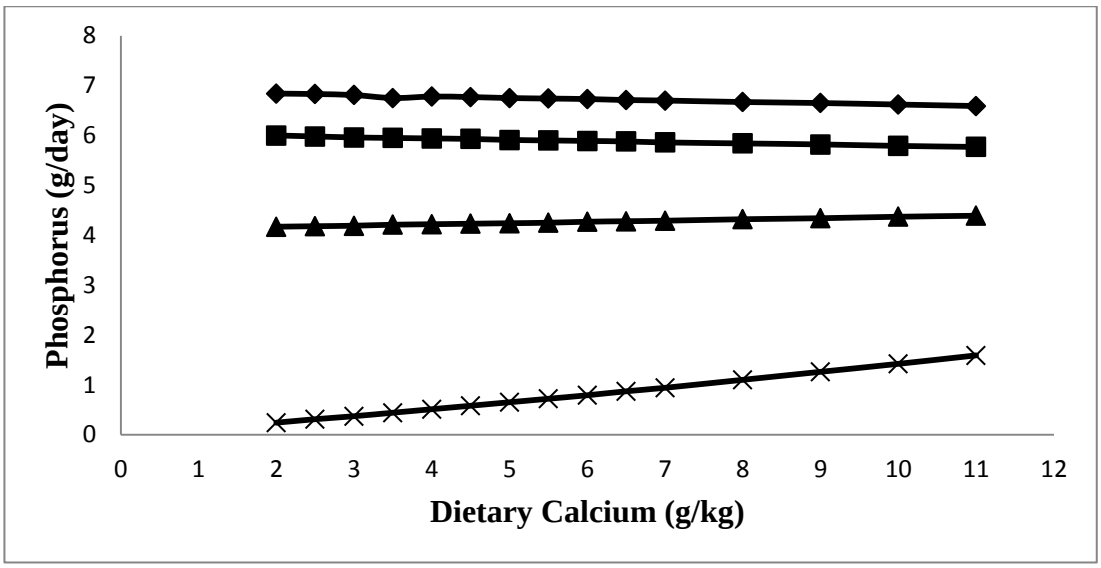


**Figure 2.8** The effect of total dietary calcium on digestible (♦) and retained (■) phosphorus (P) and hence total (▲) and insoluble (x) P excretion (g/day) for a typical UK commercial diet, supplemented with 750 FTU/kg diet *E.coli* phytase, containing 4.29 g/kg total P, separated into 2.48 phytate and 1.81 phosphate P. The diet also contained 480 FTU plant phytase. Pigs of an "Intermediate" BSAS (2003) genotype, were simulated to grow from 30-60 kg body weight.

A diet containing a constant tP at 4.29 g/kg, but containing a lower phosphate:oP ratio (i.e. is high in oP), resulted in a lower dP and hence retained P, while there was a higher P excretion and most notably oP excretion (see **Figure 2.9**). The lower digestible P in g/kg diet was due to the limited dephosphorylation by phytase enzymes. Pig requirements were met at a ratio of 1.2:1 phosphate:oP. This implies that for the default diet used that contained 4.29 total P g/kg, 2.47 g oP/kg and 1.82 g phosphate/kg, an additional 0.52 g phosphate/kg diet would be needed to meet the requirements of the default genotype.This could be achieved by the supplementation of either phosphate salts or exogenous phytase enzymes. Increasing the phosphate:oP ratio beyond 1.2 did not have an effect on P retention, while the dP increased caused more soluble P excreted through the urinary tract.

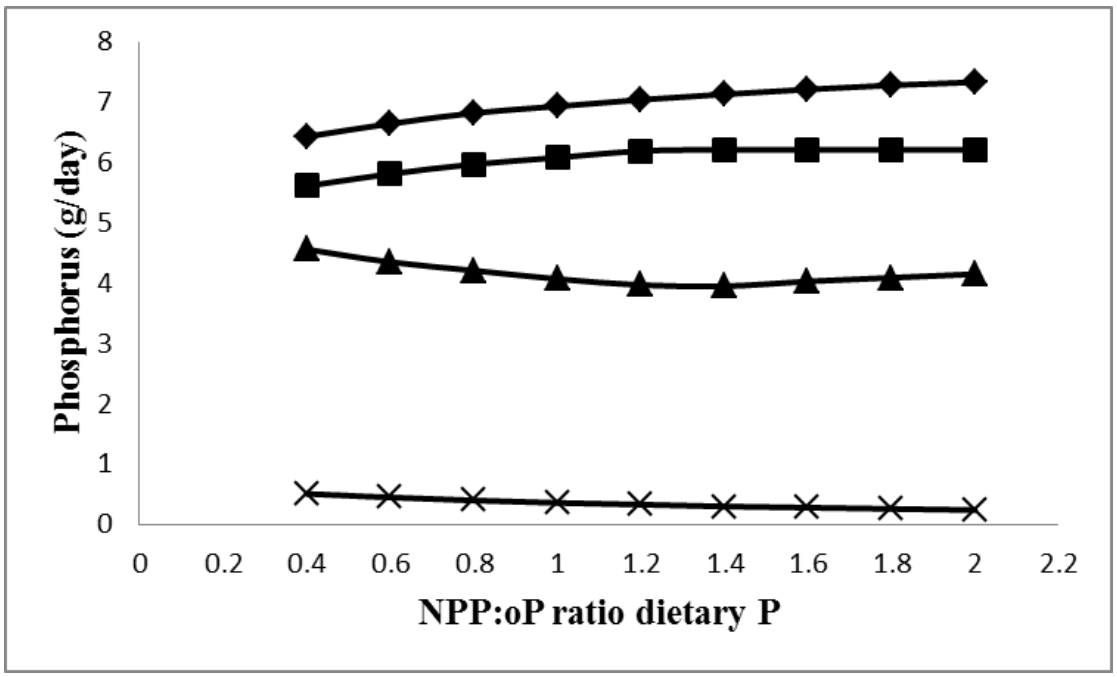


**Figure 2.9** The effect of the dietary phosphate phosphorus: phosphate ratio (NPP:oP) on digestible (♦) and retained (■) phosphorus (P) and hence total (▲) and insoluble (x) P excretion (g/day) for a typical UK commercial diet, supplemented with 750 FTU/kg diet *E.coli* phytase, containing 5.19 g/kg total Ca and 4.29 g/kg total P. The diet also contained a 480 FTU plant phytase. Pigs of an "Intermediate" BSAS (2003) pig genotype, were simulated to grow from 30-60 kg body weight.

## 2.5 Discussion

Here we address some of the assumptions underlying the model equations, the value of their parameters and their consequences on its predictions. The model is fully tested and evaluated in chapter 3. Currently there are three simulation models that deal with different aspects of P digestion and/or metabolism in the pig. The models of Fernandez (1995) and Dias et al. (2010) deal with P kinetics in the body of pigs and are based on radioactive Ca and P flows, whereas themodel of Létourneau-Montminy et al. (2011) deals with the fate of P intake in the digestive tract of growing pigs. Our model differs from these in several aspects including: (1) the ability to predict P intake, rather than dealing with P intake as an input (Fernandez, 1995; Dias et al., 2010) or dealing with it in an empirical manner (Létourneau-Montminy et al., 2011). This was achieved by the model being able to predict the food intake of pigs of different genotypes. (2) The ability to predict the fate of P in the digestive tract of pigs of different genotypes, given

access to foods of different compositions. In principle, the model of Dias et al. (2010) may be adapted to account for the consequences of P intake for different pig genotypes, although this is not made explicit by its authors. (3) A link made between P retention and the retention of other components of the body, such as protein, through isometric or allometric relationships.This is consistent with the suggestion made by NRC (2012). (4) The separate prediction of soluble and insoluble forms of P excreted in the faeces and urine of the pig, although Fernandez (1995) and Dias et al. (2010) deal with the P excreted in the faeces and urine but not the form of P excreted.

In terms of model parameterisation, there is a significant difference between the parameter values assumed by the current model and those by the model of Létourneau-Montminy et al. (2011). The latter assume that P is absorbed to the blood plasma according to both active and passive mechanisms of the gastrointestinal system. The active transport of P requires energy and it involves a sodium-phosphate co-transporter which carries two sodium for each phosphate (Gropper and Smith, 2012). The passive transport is due to the high phosphate ion in the lumen in comparison to the bloodstream, therefore it is a diffusion mechanism along the electrochemical gradient, through the intercellular junction of the small intestine (Cross et al., 1990). The model developed in this paper adopted a constant digestibility coefficient of 0.8, consistent with the suggestions of Gunther (1978) and Jongbloed (1987), thus ignoring any active P absorption that occur at very low dietary P diets (Breves and Schroder, 1991; Fernandez, 1995). The insignificance of active P absorption is supported by the study of Schulin-Zeuthen et al. (2007) and the meta-analysis of Létourneau-Montminy et al. (2012) who found that the flow of P from gut to blood was linear for a wide range of dietary P intakes. The latter estimated the P absorption to be a constant coefficient of 0.8 for phosphates. The passive flow of phosphate from the lumen to the blood-stream is also backed up by the review of France et al. (2010) who concluded that in pigs, there is little regulation of P absorbed from the lumen into the bloodstream, therefore emphasising the importance of the renal activity in regulating the P in the blood. It can be concluded that while there may be a higher digestibility due to active transport mechanisms in very low P diets, such P levels are outside the bounds of commercial pig diets.

We used first-order kinetics equations to describe the relationship of oP dephosphorylation with microbial and plant phytase enzymes (**equation 2.1**). These equations are consistent with the Michaelis-Menten kinetics, in terms of the response to different enzyme levels, when the substrate level is not very high. One benefit of the applied functions is also that they allow the determinations of the maximum proportion of the phytate that can be dephosphorylated by a given enzyme, i.e. the total amount of the 'reactive' phytate. However, it should be noted that there are limitations in the applicability of equations, which have been derived empirically. In chapter 3 it is demonstrated that the ability of the model to predict the response to phytase depends on the composition of the diet and thus on the amount of the 'reactive' phytate in diet as opposite to the total phytate content. Developing more general functions for enzyme kinetics will be a subject for future work when more relevant data becomes available.

Although there is experimental evidence that the pH of the gastrointestinal tract causes changes in the oP solubility, as well as phytase activity (Selle and Ravindran, 2008), trying to simulate diurnal changes in pH of the gastrointestinal system and especially the stomach, is a complex process. For example, in order to simulate diurnal changes in stomach pH, which in turn will affect P digestion, one would need to simulate diurnal patterns of FI and even simulate the effects of anticipatory feeding behaviour in pigs. Currently there are no available models that predict successfully the food intake of pigs at time scales shorter than a day (Black, 2009). Létourneau-Montminy et al. (2011) attempted to simulate the change of the stomach pH making assumptions about the diurnal patterns of FI. We decided to ignore the effect of stomach pH in the current model and to assume that no Ca-phytate complexes were formed there. The consequence of this could be an over-estimation of P retention and under-estimation of P excreted at elevated dietary Ca levels. The dietary Ca acts as an acid buffer, causing the stomach pH to increase thus decreasing the oP solubility available for dephosphorylation by phytase enzymes, while phytase activity also decreases (Selle and Ravindran, 2008).

The model assumes that there is a linear relationship between dietary Ca and proportion of phytate dephosphorylation in the small intestine. This has the consequence that on diets non-limiting in dP an increase in dietary Ca will not change the rate of P retained, although it will change the ratio of soluble: insoluble P excreted. However, we appreciate that the same principle may not apply when a diet low in Ca is supplemented

with Ca (Larsen et al., 2000). There is some empirical evidence thatwhen Ca is limiting, increasing its amount in diet would improve the P retention. For example Poulsen et al. (2010) found increased P utilization with increasing dietary Ca in diets with phytase addition. They concluded that diets with inadequate amount of Ca result in reduction of P utilization, as such diets are not able to support the co-deposition of P and Ca. This would also affect the amount of P excretion. Similar effect was found in an earlier study by Vipperman et al (1974), where a significant interaction between calcium and phosphorus was observed for P digestibility and retention.

In the current version of the model, the missing interaction between Ca and P retention limits the use of the model when applied with diets that are limiting in Ca. This limitation is also demonstrated by some of the comparisons with experimental data presented in chapter 3. This is clearly one of the topics where further model development is needed and for this purpose more systematic, empirical data on Ca metabolism should be generated. However, it should be noted that currently the industry is concerned with the oversupply as opposed the undersupply of Ca in pig diets.

An important assumption in the model was that the efficiency of dP utilisation for growth was independent of pig size, which is in agreement with NRC (2012). This is inconsistent with the study of Pettey et al. (2006), who suggested that the efficiency varies between 0.97-0.89 for smaller and larger pigs, respectively. The suggested decline of $e_{growth}$ with pig size is in contrast with the findings of Kemme et al. (1997), who have shown exactly the opposite. There are other estimates of $e_{growth}$ in the literature (Schulin-Zeuthen et al., 2007), suggesting that its value is much lower closer to 0.7. As the latter value was estimated from data meta-analysis, it is likely that it includes estimates of both gross and net efficiency. The above inconsistencies in the literature do not allow for systematic conclusions to be drawn on the effect of pig size on the efficiency of P utilisation above maintenance. These inconsistencies cause a source of uncertainty in the models outputs, with 9% and 3% change in the P retained when the efficiency of dP utilisation for growth changed to 0.98 and 0.87, respectively, see chapter 3. Due to the mechanistic nature P utilisation by the model, the model has the potential to be converted to account for a different efficiency of dP utilisation for growth. We have further assumed that the value of $e_{growth}$ would be constant across genotypes and sexes in analogy to what has been suggested by Sandberg et al. (2005)

for the net efficiency of protein utilisation in pigs. Again this assumption has yet to be addressed directly in the literature (Kyriazakis, 2011).

The model links P retention to the retention of body protein and hence to current protein mass, at any stage of growth, through simple allometric or isometric relationships. In addition, digestible P requirements both for maintenance and maximum retention are also functions of Pr mass. Our approach has several advantages, including the description of the genotype in the simple terms described by Emmans and Kyriazakis (2001) and applied to pigs by Wellock et al. (2003). We appreciate that there would be certain dietary conditions, when the allometric or isometric relationships between body P and another body components will be disturbed, as discussed above.

The current model and that of Létourneau-Montminy et al. (2011) are the only ones able to predict the different forms of P excreted from the ileum to the large intestine. Our model also predicts the fate of the oP and phosphate in the large intestine, thus it is able to predict the different forms of P excretion in the faeces. Létourneau-Montminy et al. (2011) used data on phytate ingestion to add three sub-flows from the gastro-intestinal system to blood, representing inorganic P, dietary phytate P and dietary phosphate. P excreted in faeces was apportioned similarly. The approach followed in our model is similar, but we have taken it a step further and expanded it by considering the fate of the undigested P in the large intestine. By doing so the total soluble and insoluble P excreted could be predicted, as the quantification of the fate of undigested P in the large intestine made the predictions of P excretion more accurate. Literature (Sandberg et al., 1993) clearly suggests that the microflora of the large intestine plays a significant role in the degradation of oP and the solubilisation of P excreted and that this is affected by dietary Ca that reaches the large intestine. High dietary Ca from supplemental calcium carbonate increased colonic pH, which in turn reduces the degradation of phytate in the colon (Sandberg et al., 1993). The ability of the model to predict P excreted into soluble and insoluble P makes it a valuable tool in formulating diets that minimize the excretion of soluble P, rather than just total P. Soluble P is of higher contributor to eutrophication, rather than the relatively inert insoluble P in the form of oP and its complexes.

## 2.6 Conclusion

A dynamic, deterministic model has been developed to account for the different forms of P in a pig diet and their fate through the gastrointestinal tract. The model is able to predict the intake, digestion, retention and ultimately P excretion in pigs offered access to diets of different compositions and account for the different form of P excreted. The model also takes into account the effect of pig genotype on maximum P retention and is the first of its kind in being able to predict P intake of pigs of different genotypes offered access to feeds of different composition. Some of the uncertainties associated with the assumptions made and the values of the model parameters have been discussed above. Chapter 3 deals with model evaluation, by comparing how well the model is able to predict the outcomes of experiments that deal with the issue of P retention and excretion.

## 2.7 Implications

Currently there is some disagreement about the P requirements of pigs of different genotypes and how digestible P contents of pig diets are calculated, especially for diets that include different amounts of phytate and non-phytate P. Achieving a balance between meeting digestible P requirements for optimum growth and health, and avoiding excess P intake would lead to a reduction in diffuse P levels in manure and effluents, and environmental impact from pig systems. A simulation model that predicts P intake, digestion, retention and excretion is the first, necessary step towards achieving this aim.

# Chapter 3. Modelling phosphorus intake, digestion, retention and excretion in growing and finishing pigs: model evaluation

## 3.1 Abstract

A deterministic, dynamic model was developed, to enable predictions of phosphorus (**P**) digested, retained and excreted for different pig genotypes and under different dietary conditions. Before confidence can be placed on the predictions of the model, its evaluation was required. A sensitivity analysis of model predictions to ±20% changes in the model parameters was undertaken using a basal UK industry standard diet and a pig genotype characterized by BSAS (2003) as being of 'intermediate growth'. Model outputs were most sensitive to the values of the efficiency of digestible P utilization for growth and the non-phytate P absorption coefficient from the small intestine into the bloodstream; all other model parameters influenced model outputs by less than 10%, with the majority of the parameters influencing outputs by less than 5%. Independent data sets of published experiments were used to evaluate model performance based on graphical comparisons and statistical analysis. The literature studies were selected on the basis of the following criteria: they were within the body weight range of 20-120 kg, pigs grew in a thermo-neutral environment; and they provided information on P intake, retention and excretion. In general, the model predicted satisfactorily the quantitative pig responses, in terms of P digested, retained and excreted, to variation in dietary inorganic P supply, Ca and phytase supplementation. The model performed well with 'conventional', European feed ingredients and poorly with 'less conventional' ones, such as dried distillers grains with solubles and canola meal. Explanations for these inconsistencies in the predictions are offered in the paper and they are expected to lead to further model development and improvement. The latter would include the characterisation of the origin of phytate in pig diets.

## 3.2 Introduction

In the previous chapter, a deterministic, dynamic model, which accounts for the digestibility of dietary phosphorus (**P**) by growing and finishing pigs, and its fate as

retained and excreted P, was developed. The model enables the prediction of the effects of pig genotype and its interaction with diet on P retention, as well as the prediction of the form of P excreted, as soluble and insoluble P. These are important advances over existing models that predict P digestion (Létourneau-Montminy et al., 2011) or P retention (Fernandez, 1995; Dias et al., 2010). The developed model allows for the simultaneous testing of a range of variables, and enables the formulation of diets with high P digestibility, whilst supplying digestible P that closely matches pig requirements; therefore it enables minimisation of P excreted.

Model behaviour was consistent with our current understanding of P digestion and retention, see chapter 2. When compared to BSAS (2003) nutrient requirement standards, our model moderately overestimated digestible P requirements for growing pigs, while for finishing pigs the same requirements were moderately underestimated. The differences in estimated requirements may reflect differences in the methodology, and as a result before confidence can be placed on the predictions of the model, this needs to be evaluated. The scarcity of appropriate studies identified during model development resulted in an inherent uncertainty for the values of a number of model parameters. A sensitivity analysis of the predictions to changes in the main model parameters needed to be undertaken and this was the first aim of this paper. The second aim was to qualitatively and quantitatively compare model predictions with observations from the literature that were not used during model parameterization. The wider the circumstances under which model predictions can be tested, the more confidence can be applied on the appropriateness of the model concepts, the accuracy of parameters upon which it is based and the relevance of its predictions (Black, 1995).

## 3.3 Material and methods

### *3.3.1 Sensitivity analyses*

A reference pig genotype and diet were chosen as the starting point for the sensitivity analysis. The reference diet used is a typical grower diet currently in use by the UK pig industry with an analysed chemical composition of: 9.6 MJ Net Energy, 172.5 g crude protein, 11.07 g lysine, 5.19 g total Ca and 4.29g total P per kg of diet as fed, was used

for this purpose. The dietary total P consisted of 2.47 g phytate (**oP**) and 1.82 g phosphate (**NPP**) /kg diet. This default diet consisted of wheat- 600 g/kg, barley- 110 g/kg, rapeseed meal- 80 g/kg, soybean meal- 77g/kg and sunflower meal- 50 g/kg fresh diet. The incorporation of wheat made the diet relatively high in plant phytase at 480 FTU/kg. As the feed was assumed to be pelleted, some denaturation of the plant phytase activity was expected (Jongbloed and Kemme, 1990). The diet was supplemented with an extra 750 FTU *Escherichia coli* microbial phytase according to current industry practice. The reference (default) pig genotype used in the sensitivity analysis was characterized by BSAS (2003) as being of 'intermediate growth' with 40 and 48 kg protein (**$Pr_m$**) and lipid (**$L_m$**) at maturity respectively, and a 0.01175 Gompertz growth rate parameter (per day). The *in silico* pig, was simulated to grow from 30 kg to 60 kg BW and was given an *ad libitum* access to food and water.

Sensitivity analysis is an integral part of model development and involves the analytical examination of input parameters to aid in model validation and provide guidance for future research. A sensitivity analysis was undertaken to evaluate the effect of variation in the values of the model parameters given in **Table 3.1** on model outputs, in terms of P retained, total P and soluble P excreted. There are several methodologies for conducting parameter sensitivity analysis, the most common being the one-at-a-time sensitivity measures and the factorial analysis (Hamby, 1994).

The one-at-a-time methodology has been chosen, because it is the simplest approach to conceptualize, where sensitivity measures are determined by varying each parameter independently while all others are held constant. The sensitivity measure was determined by adjusting parameter values by a percentage of their base-case value (±20%, ±SD). Varying the input parameter by a standard amount of ±20% is justified because the majority of the parameters have approximately 20% standard deviation as was seen from the previous chapter. This methodology for sensitivity analysis is widely used in nutritional models (Halas et al., 2004; Vagenas et al., 2007). The one-at-a-time methodology is more preferable than the factorial methodology due to the high number of parameters that needs to be tested. A factorial analysis involves choosing a given number of samples for each parameter and running the model for all combinations of the samples (Box et al., 1978; Rose, 1983). A large number of parameters quickly prohibit a thorough examination of the model because of the large number of model runs required (Hamby, 1994). A more accurate test of local sensitivity examines the

change in output as each parameter is individually increased by a factor of its standard deviation (Hamby, 1994).

The sensitivity analysis was performed using ±20% change in the investigated default value parameter, while keeping all other parameters constant. The only exceptions were the analysis for the effects of the efficiency of P utilisation ($\mathbf{e_{growth}}$) and the maximum oP dephosphorylation by endogenous large intestine phytase ($\mathbf{K_{max.LI}}$), since their default values were 0.94 and 1, respectively. The excreted soluble P is the desired trait to measure, because water soluble P excretion represents the highest potential risk for losses by runoff in agricultural fields causing eutrophication (Maguire et al., 2005).

**Table 3.1** Abbreviation, descriptions, default values (derived from chapter 2) and units of parameters used by the model.

| Abbreviation | Default Value | Description |
|---|---|---|
| $e_{growth}$ | 0.940 | Efficiency of P utilization for growth |
| $K_{abs.NPP}$ | 0.800 | Parameter constant at which phosphate P is absorbed from the lumen of the small intestine to the blood-stream for retention |
| $Kmax._{A.niger}$ | 0.562 | Maximum of phytate dephosphorylation by *A. niger* phytase (g/g) |
| $Kmax._{E.coli}$ | 0.532 | Maximum of phytate dephosphorylation by *E. coli* phytase (g/g) |
| $Kmax._{LI}$ | 1.000 | Maximum of phytate dephosphorylation by endogenous large intestine phytase (g/g) |
| $Kmax._{Plant}$ | 0.377 | Maximum of phytate dephosphorylation by plant phytase (g/g) |
| $Kmax._{SI}$ | 0.260 | Maximum of phytate dephosphorylation by endogenous small intestine phytase (g/g) |
| $K_{pellet}$ | 0.500 | Parameter constant for the ratio of phytate dephosphorylation through the exposure to high temperature |
| $R_{A.niger}$ | 0.001 | Rate of phytate dephosphorylation by microbial *A. niger* |

| | | |
|---|---|---|
| | | phytase (g/day) |
| $R_{E.coli}$ | 0.002 | Rate of phytate dephosphorylation by microbial *E.coli* phytase (g/day) |
| $R_{LI}$ | 0.076 | Rate of phytate dephosphorylation by large intestine phytase (g/day) |
| $R_{Plant}$ | 0.002 | Rate of phytate dephosphorylation by plant phytase (g/day) |
| $R_{SI,Phy}$ | 0.016 | Rate of phytate dephosphorylation by small intestine phytase (g/day) |

### *3.3.2 Model evaluation*

Independent data sets of published experiments were used to evaluate model performance based on graphical comparisons and statistical analysis. Model performance was evaluated on the basis of the goodness of fit of the observed against the predicted P digested, P retained, total and soluble P excreted as g/day. The literature studies selected for evaluation purposes were based on the following criteria: (1) they used growing-finishing pigs within the range of ~20 to 120 kg body weight (BW); (2) pigs grew in a thermo-neutral environment and no environmental stressors were assumed to be operating (Wellock et al., 2004); and (3) the studies provided information at least on P intake, digestible and excreted P in the faeces, and when possible information on retained P, total and soluble P excreted. Preference was given to studies that contained more than one treatment in addition to the control, as this allowed for systematic exploration of the model.

Studies that met the above criteria were used in the evaluation process to test for the effect of: (1) inclusion of inorganic P (Ekpe et al., 2002*;* Lopes et al., 2009*)*; (2) different levels of phytate in the diet (Trujillo et al., 2010); (3) *Aspergilus niger* and *E.coli* phytase supplementation (Akinmusire and Adeola, 2009; Jendza and Adeola, 2009; Poulsen et al., *2010;* Trujillo et al., 2010; Almeida and Stein, 2012); and (4) different levels of dietary Ca (Poulsen et al., 2010; Stein et al., 2010).

Ekpe et al. (2002) investigated the effects of increasing dietary di-calcium phosphate levels on P digestibility, retention and excretion. The total P excretion was separated into faecal and urinary P, since urine and faeces were collected and analysed separately. A total of 20 crossbreed barrows, C15 sows x Canabrid boars, (n=4 per treatment) with a BW of 54 kg received one of five dietary treatments: 0, 4.8, 9.7, 14.5 and 19.4 g/kg diet di-calcium phosphate, whilst keeping the total Ca content of the diet constant at 9 g/kg. The oP and phosphate content of the diet were not analysed, neither was there a chemical analysis of the main ingredients used. Pigswere allowed *ad libitum* access to the diets.

Lopes et al. (2009) like Ekpe et al. (2002), evaluated the effect of increasing di-calcium phosphate: The main difference between the two experiments was in the experimental

methodology. Lopes et al. (2009) used radio-isotopic kinetics and investigated actual P absorbed from the lumen of the gastrointestinal tract to the bloodstream, as opposed to the total collection method. The study of Lopes et al. (2009) provided an important validation for the P digestion module of the model. A total of 10 crossbred (no specified breed) barrows (n=2 per treatment) with a mean BW of 20kg, received one of five dietary treatment: 0, 5.2, 10.5, 17.5 and 21.8 g/kg diet di-calcium phosphate, while keeping the total Ca content of the diet constant at 6 g/kg. The feed allowance was offered twice daily. The oP and phosphate contents were not analysed, but estimated using INRA feed tables (Sauvant et al., 2004).

Trujillo et al. (2010) investigated the effect of different levels of oP through the supplementation of rice bran, with or without supplementation of 750 FTU of microbial *Aspergillus niger* phytase at the highest and lowest levels of oP. The phosphates of the diets were relatively constant at 1.3 (±0.2) g/kg diet and so was the total dietary Ca at 5.59 (±0.8) g/kg diet. Twenty-four crossbred (Yorkshire x Landrace) x Hampshire barrows with a 87.5 (±2.51) kg BW (n=4 per treatment) were used in the experiment. To prepare the experimental diets, 0, 75, 150 and 300 g/kg of the basal corn-soybean meal based diet was replaced with equivalent amounts of rice bran.The phytate and non-phytate contents of the diets were not analysed, neither was there a chemical analysis of the main ingredients. No inorganic P was supplemented to the diets, while the total Ca and P were analysed for each diet. P digestibility was assessed by the total collection method. The pigs were offered food at 3% of BW.This experiment allowed the comparison of model behaviour to changes in the oP content of the diet and microbial phytase on P retained and excreted.

Akinmusire and Adeola (2009) studied the effect of different inclusion levels (0-500 g/kg diet) of canola or soybean meals supplemented to semi-purified diets, with and without the supplementation of 1000 FTU *Escherichia coli* phytase on P digestibility, for 17 kg BW pigs. A total of forty-eight and thirty-six barrows, of no specified breed, were used with n=8 and n=6 per treatment for canola and soybean meal, respectively. The feed allowance was based on the individual BW of the pigs. A total apparent P digestibility was calculated by analysing the collected faeces. The authors analysed the nutrient composition of the canola and soybean meal (g/kg), and thus accurate phytate and non-phytate contents of the diets were available. The actual phytase activity /kg for

each diet had also been analysed. The dietary Ca level also increased by the supplementation of either the canola or soybean based meal.

Almeida and Stein (2012) studied the effect of four levels of microbial *Escherichia coli* phytase supplementation, ranging from 0-1100 FTU, to corn-, dried distillers grains with solubles (DDGS)-, high P dried distillers grains (HP DDG)- and corn germ- based diets. We have concentrated upon the consequences of the better known corn and DDGS based diets. A total of 48 crossbreed Large White x Landrace pigs, (n=6 per treatment) were fed either the corn- or DDGS -based diets and received one of four levels of phytase inclusion per ingredient. Almeida and Stein (2012) also used a P-free diet in order to measure basal endogenous P losses. The study also measured the phytate and non-phytate P contents of each feed ingredient, and the total dietary Ca content was constant for the corn-based diets at 5.2 g/kg, while the dietary Ca of the DDGS-based diets increased with phytase supplementation.

Jendza and Adeola (2009) tested for the effects of graded levels of microbial phytase enzymes (ranging from 0 – 1000 FTU) on P digested and retained for two pig BW. There were 6 barrows per treatment with an average initial BW of either 20 or 51 kg. Pigs received two equal feed allowances daily with average daily feed intakes set at 4.0 and 3.7% of the initial BW, respectively. The oP and phosphate contents of the diet were not analysed, but estimated using INRA feed tables (Sauvant et al., 2004). The total Ca content of the diet was constant at 6.5 g/kg. It is important to note that Jendza and Adeola (2009), as well as measuring total P excreted (g/day), also measured water soluble P excretion. Because the experiment suggested that phytase inclusion had a minimal effect on P digestibility at high BW, data from the latter were not considered further.

Stein et al. (2011) determined the effect of variation in different dietary Ca levels on P digestibility, retention and excretion separated into faecal and urinary P g/day excreted. A total of 36 crossbred barrows with a BW of 23.1 (±4.4) kg, with six barrows per treatment received one of the six dietary treatments: 3.3, 4.6, 5.1, 6.7, 9.2 and 10.4 g/kg Ca achieved by supplementation with calcium carbonate. The oP and phosphate content of the diet remained constant. The study analysed only the total P content of the diet, while the oP:phosphate ratio of the diets was calculated. The calcium carbonate used in the experiment was analysed and contained 38.83% Ca. Pigs were fed the experimental

diets at approximately 3 times their maintenance requirements for energy. Apparent P digestibility was assessed by a total collection method.

Poulsen et al. (2010) evaluated the effect of dietary Ca content with and without microbial *A. niger* phytase supplementation in a diet with high intrinsic phytase, due to the high concentration of wheat in the diet. The diet consisted of barley, wheat and soybean meal, and was not supplemented with any inorganic P. The study was conducted on 48 pigs (no specified breed) weighing 38.9 (±1.9) kg BW. The three dietary Ca levels investigated were measured to be 4, 6, and 8 g/kg diet, with or without 750 FTU phytase supplementation, and with the diet plant phytase activity being 650 FTU. Diet analyses also took place to estimate the total P and Ca content as well as the phytate content in g/kg dry matter. The study did not specify how the pigs were fed, but provided intakes of total P and Ca.

The observed feed intakes of the above experiments were treated as inputs to the model. In the few occasions where pigs were fed above their requirements, as in the experiments of Ekpe et al. (2002), the pig genotype values were adjusted in order to treat the maximum P retention as an input, in the manner described by Wellock et al. (2003). When the studies used did not provide the analyzed oP and phosphate contents of their diets, then their oP and phosphate contents were estimated based on the INRA feed tables (Sauvant et al., 2004).

The statistical package MODEVAL v1.1 developed by Smith et al. (1997) was used for the purposes of model evaluation in a series of statistical tests to assess their goodness-of-fit. (1) The correlation coefficients (***r***) is used to assess whether simulated values followed the same pattern as observed values, with the value of unity being the best fit. (2) The coefficient of variation for the root mean square error (CV-**RMSE**) measures how close the predicted measurements are to the observed values. The statistical significance of CV-RMSE was then assessed by CV-$RMSE_{95\%}$. A RMSE value greater than CV-$RMSE_{95\%}$ suggests that the predicted values are not within the 95% confidence intervals of the observed data. (3) The relative error (**E**) determines the bias of the predicted results, which is the total difference between predictions and observations:

$$E = \frac{\sum^{\frac{(O_i - P_i)}{O_i}}}{n} \times 100\% \quad (3.1)$$

where, $E$ is the relative error (%), $O_i$ is the observed value, $P_i$ is the predicted value and $n$ is the number of observations. The closer to zero the E value is, the less bias exists between predicted and observed results. A positive E value indicates under-estimation by the predictions and the opposite is the case for negative E values. The statistical significance of E was then assessed with $E_{95\%}$. An E value less than $E_{95\%}$ indicates that the simulated values fell within the 95% confidence interval of the measurements.

## 3.4 Results

### *3.4.1 Sensitivity analyses*

The results of the sensitivity analyses on retained P and total, soluble and insoluble P excreted are presented in **Table 3.2**.

Varying model parameters generally had small overall effects on P outputs, the exception being the $e_{growth}$ and the absorption coefficient of the phosphate from the SI lumen ($K_{abs.NPP}$) parameters. A decrease of 20% in the $e_{growth}$ parameter resulted in approximately 20% decrease in the P retained and 20% increase in the total and soluble P excreted. A 20% decrease in $K_{abs.NPP}$ resulted in a 13% decrease in P retained, as well as 13% and 17% increase in the total and soluble P excreted, respectively. There was a smaller magnitude of change for the 20% increase in the $K_{abs.NPP}$ parameter, compared to the 20% decrease. Smaller effects originated from the change in the parameter that defined the maximum oP dephosphorylation by endogenous microbial large intestine phytase ($K_{max\ LI}$); a reduction of 10% was achieved when this parameter decreased by 20% from its default value. On the other hand, the change in the maximum value of oP dephosphorylation by *E. coli* phytase ($K_{max\textit{E.coli}}$) had a relatively small impact on the P retained and total P excreted. All other parameters investigated had a less than 4% effect on model outputs when their values changed by ± 20%.

**Table 3.2** Output of the sensitivity analyses performed when a specific model parameter was increased or decreased by 20%.

| | Retained P | | | Total P excreted | | | Soluble P excreted | | |
|---|---|---|---|---|---|---|---|---|---|
| Parameter | Default (g/day) | 0.8 × default (%) | 1.2 × default (%) | Default (g/day) | 0.8 × default (%) | 1.2 × default (%) | Default (g/day) | 0.8 × default (%) | 1.2 × default (%) |
| $e_{growth}$ | 5.28 | −19.9 | 6.6# | 4.51 | 17.8 | −5.5# | 3.79 | 25.0 | −8.3# |
| $K_{abs\ NPP}$ | 5.28 | −12.7 | 8.5 | 4.51 | 12.6 | −8.4 | 3.79 | 16.5 | −12.6 |
| $K_{max\ E.\ coli}$ | 5.28 | −5.3 | 5.1 | 4.51 | 6.4 | −6.1 | 3.79 | 1.6 | 1.8 |
| $K_{max\ LI}$ | 5.28 | 0.0 | 0.0 | 4.51 | 0.0 | 0.0 | 3.79 | −9.5 | N/A |
| $K_{max\ Plant}$ | 5.28 | −1.5 | 1.7 | 4.51 | 2.0 | −1.8 | 3.79 | 0.6 | −0.5 |
| $K_{max\ SI}$ | 5.28 | −1.9 | 2.2 | 4.51 | 2.4 | −2.6 | 3.79 | 0.6 | −0.9 |
| $K_{max\ A.\ niger}$ | 4.91 | −4.3 | 4.3 | 4.89 | 4.1 | −4.3 | 3.96 | 1.2 | −1.2 |
| $K_{pellet}$ | 5.28 | −1.5 | 1.7 | 4.51 | 2.0 | −1.8 | 3.79 | 0.6 | −0.4 |
| $R_{SI}$ | 5.28 | 0.6 | −0.6 | 4.51 | −0.7 | 0.9 | 3.79 | 0.0 | 0.3 |
| $R_{A.\ niger}$ | 4.91 | −3.1 | 2.9 | 4.89 | 3.1 | −2.9 | 3.96 | 0.9 | −0.7 |
| $R_{E.\ coli}$ | 5.28 | −2.7 | 2.5 | 4.51 | 3.3 | −3.0 | 3.79 | 0.9 | −0.9 |
| $R_{LI}$ | 5.28 | 0.0 | 0.0 | 4.51 | 0.0 | 0.0 | 3.79 | 3.6 | −3.9 |
| $R_{Plant}$ | 5.28 | −0.8 | 0.9 | 4.51 | 1.1 | −0.9 | 3.79 | 0.3 | −0.3 |

Default outputs of the analyses performed were for a pig simulated to grow between 30 and 60 kg BW, having an 'Intermediate' BSAS (2003) pig genotype and offered a typical UK commercial diet. The sensitivity analysis outputs on retained P, total and soluble P are shown as the % change from the default values (**Table 3.1**).

# The default value of the efficiency of digestible P utilization was set at 0.94% and a 20% increase of this parameter was not possible, therefore it was set as unity.

### *3.4.2 Comparison of the model with published trials*

#### *3.4.2.1 The effect of increasing dietary levels of inorganic P*

The graphical comparison between the experimental observations and the model predictions for Ekpe et al. (2002) are shown in **Figure 3.1**, whilst their statistical comparisons are presented in **Table 3.3**. P retained increased with increasing inorganic P supplementation in a linear-plateau manner, while the total P excreted increased at a faster rate, once maximum P retained was achieved. The simulated values followed the same pattern as the observed results, with correlation coefficients of 0.88 and 1.00 for P retained and total P excreted, respectively. The predicted measurements were very close to the observed results, as shown by the low CV-RMSE for both P retained and excreted, which were smaller than the CV-RMSE$_{95\%}$, indicating that the simulated values fell within the 95% confidence interval of the measurements. An E value lower than $E_{95\%}$ also indicated that there was no bias in the simulations; as they did not consistently over or under-estimate the predicted results compared to the observed values.

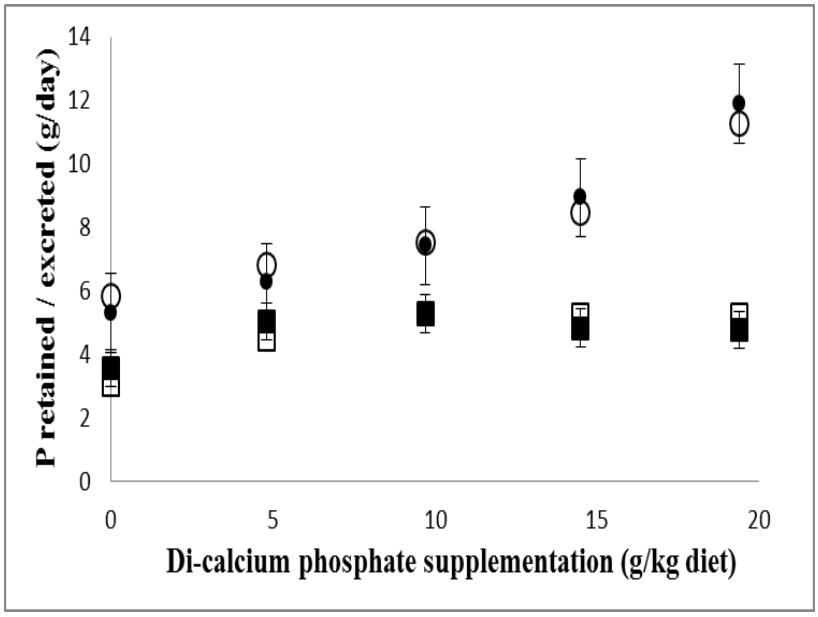


**Figure 3.1** Comparison of experimental observations and their standard error of the mean (**SEM**) (■,●) by Ekpe et al. (2002) to simulated predictions (□,○) for retained phosphorus (■,□) and total phosphorus (tP) excreted (●,○). Ekpe et al. (2002) evaluated the effect of increasing di-calcium phosphate (iP) in pig diets; for details of the diets see Materials and Methods.

**Table 3.3** The outcomes of the statistical analyses used to assess the goodness-of-fit between the simulated predictions against observed outcomes of experiments reported in the literature.

| | r[1] | CV-RMSE[2] | CV-$RMSE_{95}$[3] | E[4] | $E_{95}$[5] |
|---|---|---|---|---|---|
| Ekpe et al. (2002) | | | | | |
| Retained P | 0.88 | 9.54 | 40.05 | 1.48 | 40.83 |
| Total P excreted | 1.00 | 6.24 | 49.16 | -1.85 | 53.16 |
| Lopes et al. (2009) | | | | | |
| Digested P | 0.98 | 16.05 | - | 10.90 | - |
| Total P excreted | 0.90 | 18.15 | - | -15.39 | - |
| Trujillo et al. (2010) | | | | | |
| Retained P | 0.77 | 18.82 | 42.53 | -7.89 | 44.73 |
| Retained P +750 FTU | N/A | 28.94 | 27.97 | 16.53 | 33.88 |
| Total P excreted | 0.99 | 5.72 | 8.55 | 1.57 | 9.35 |
| Total P excreted +750 FTU | N/A | 6.21 | 10.53 | -4.58 | 11.97 |
| Akinmusire & Adeola (2010) | | | | | |
| Faecal P excretion:Canola diet | 1.00 | 40.17 | 118.38 | 38.96 | 141.14 |
| Faecal P excretion Canola + 1000 FTU | 1.00 | 14.93 | 213.35 | 16.96 | 244.91 |
| Faecal P excretion:Soybean meal diet | 0.99 | 4.82 | 173.98 | -2.64 | 204.76 |
| Faecal P excretion Soybean + 1000 FTU | 1.00 | 20.93 | 303.84 | 14.82 | 357.17 |
| Almeida & Stein (2012) | | | | | |
| Faecal P excreted from Corn meal | 0.62 | 18.49 | 56.43 | -7.37 | 58.40 |
| Faecal P excreted from DDGS meal | 0.98 | 37.43 | 57.37 | -37.94 | 58.06 |
| Jendza & Adeola (2009) | | | | | |
| Total P excreted | 1.00 | 8.10 | 102.67 | -7.61 | 103.53 |
| Water soluble P excreted | 0.95 | 11.88 | 73.30 | 11.46 | 73.86 |
| Stein et al. (2010) | | | | | |
| Retained P | 0.48 | 12.48 | 46.00 | -7.34 | 46.45 |
| Total P excreted | 0.25 | 12.76 | 19.60 | -10.27 | 19.76 |
| Poulsen et al. (2010) | | | | | |
| Retained P | 0.42 | 3.53 | 121.20 | -2.87 | 121.23 |
| Retained P + 750 FTU | -0.75 | 13.24 | 90.22 | -3.40 | 91.80 |
| Total P excreted | 0.69 | 2.29 | 101.10 | 1.86 | 101.82 |
| Total P excreted + 750 FTU | -0.96 | 14.34 | 128.81 | -0.18 | 130.78 |

[1] *r* : the correlation coefficient

[2] CV-RMSE : the coefficient of variation of the root mean square error

[3] $CV\text{-}RMSE_{95}$ : the 95% confidence interval of CV-RMSE

[4]E : the relative error

[5]$E_{95}$ : the 95% confidence interval of E

The experiment of Lopes et al. (2009) revealed that the P absorbed from the lumen of the small intestine into the bloodstream responded linearly to dicalcium phosphate supplementation and the response was in good agreement with the predicted results (**Figure 3.2**). The correlation between observed and predicted results for P digested and excreted were high, with correlation coefficients of 0.90 and 0.98, respectively (**Table 3.3**). The predicted measurements and observed results were close to each other and had a low CV-RMSE. Nevertheless, despite the low CV-RMSE, there was a high E, indicating that there was a bias in the predicted results. It can be seen from **Figure 3.2** that there was an under-estimation of the P digested and an over-estimation of the total P excreted; this was particularly the case for one of the treatments. Lopes et al. (2009) did not provide standard errors of the observed results, therefore the $RMSE_{95\%}$ and $E_{95\%}$ could not be calculated.

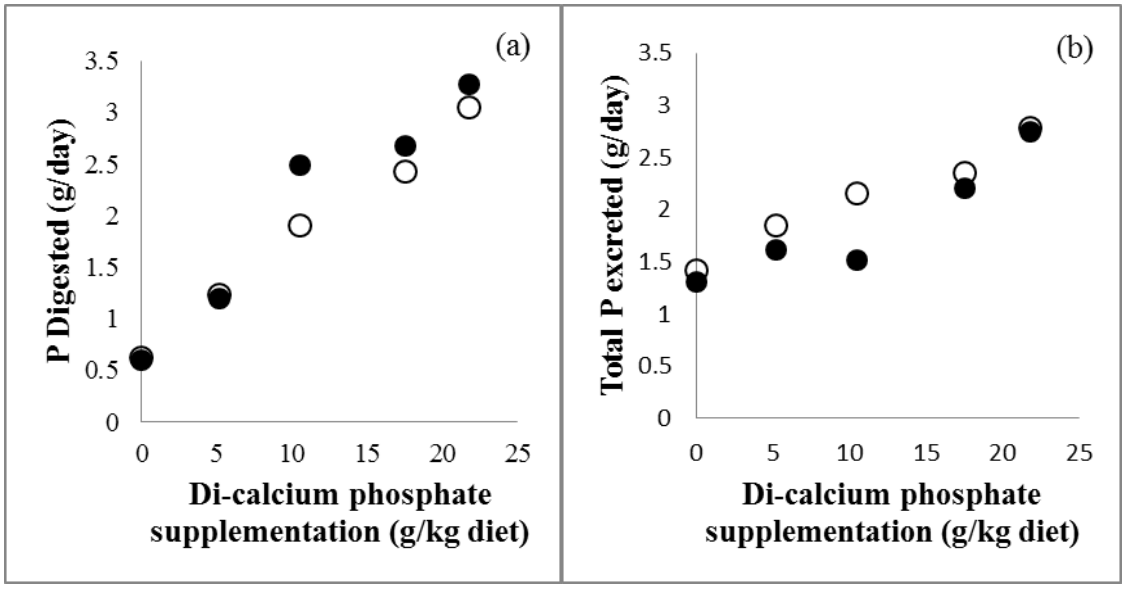


**Figure 3.2** Comparison of experimental observations (●) by Lopes et al. (2009) to simulated predictions (○) for **(a)** digested phosphorus and **(b)** total phosphorus excreted. Lopes et al. (2009) evaluated the effect of increasing di-calcium phosphate using a radio isotopic experiment.

### *3.4.2.2 The effect of different levels of phytate in the diet*

Trujillo et al. (2010) investigated the effect of different levels of phytate with or without the supplementation of 750 FTU of microbial *A.niger* phytase. The P retained and total P excreted without phytase supplementation increased with increasing phytate content of the diet, with the rate of the increase being similar between observed values and predicted results (**Figure 3.3**and **Table 3.3)**. The correlation coefficient (0.99) between observed and predicted values suggested that the total P excreted was predicted well. However, the correlation coefficient for retained P was lower (0.77). The predicted P retained also had three times higher CV-RMSE than the CV-RMSE for total P excreted. Despite the larger CV-RMSE in P retained, the low E value indicated that there was no bias in the predictions, as there was no consistent over or under-estimation of the predicted results. As far as supplementation of 750 FTU phytase was concerned, the P retained for phytase supplementation, had 4.7 times higher CV-RMSE, 29%, as opposed to 6% for the total P excreted. The bias in the predictions was high for P retained when phytase was supplemented, with the predicted values being consistently under-estimated.

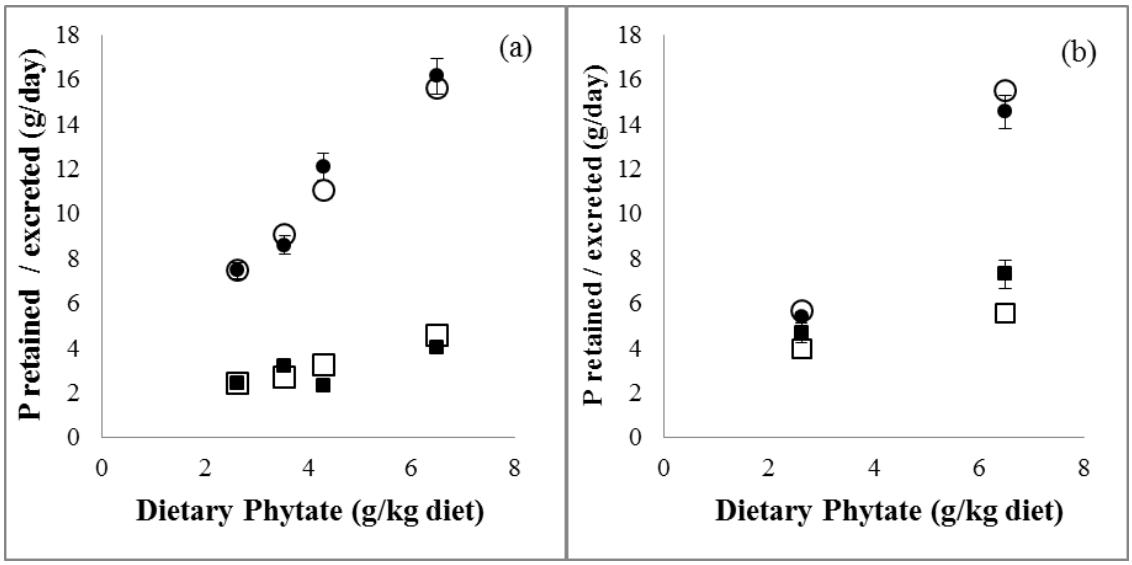


**Figure 3.3** Comparison of experimental observations and their SEM (■,●) by Trujillo et al. (2010) to simulated predictions (□,○) for retained phosphorus (■,□) and total phosphorus (tP) excreted (●,○). Trujillo et al. (2010) investigated the effects of different levels of phytate through the supplementation of rice bran, **(a)** without and **(b)** with 750 FTU of microbial *A. niger* phytase.

### *3.4.2.3 The effect of phytase supplementation*

Akinmusire and Adeola (2009) investigated the effect of different levels of canola and soybean meals, with and without the supplementation of 1000 FTU *E. coli* phytase. From the graphical analysis of the results in **Figure 3.4**, it can be seen that the model predicts with high accuracy the faecal P excreted with and without phytase supplementation for the soybean based diets. There was a very high correlation coefficient and low CV-RMSE between predicted and observed results for all these (**Table 3.3**). Nevertheless, the statistical analysis revealed that there was a bias in over-estimating the faecal P excreted when phytase was supplemented, as the E value was high (14.82%). Whilst the model predicted accurately the faecal P excreted from the soybean based diets, the model failed to predict accurately the response of faecal P excreted when the pigs were fed the canola-based diets. Despite the very high correlation coefficient between predicted and observed results for the canola based diets (both with and without phytase supplementation), there was a very high CV-RMSE between predicted and observed results especially in the diets without phytase supplementation. The latter values in combination with the high relative error (E) suggested that there was a consistent and significant under-estimation of the predicted P excreted by the model for canola-based diets.

Almeida and Stein (2012) examined the effect of graded levels of microbial *E.coli* supplementation on corn and DDGS based diets. The graphical comparison in **Figure 3.5** illustrates that when compared to the observed data, the simulated results of faecal P excretion for corn based diet were in closer agreement to the observed values than for the DDGS based diets. **Table 3.3** shows that the corn based diet simulations had half the CV- RMSE compared to the DDGS based diet simulations. The lower CV-RMSE, implied closer predictions to the observed values for corn based diets. Similarly, the simulated values for the corn based diet had lower E values, indicating an absence of bias, while the opposite was the case for the values of the DDGS based diets. Nevertheless, the statistical analysis also revealed that the simulated values of the corn based diet did not follow the same pattern as the observed values ($r = 0.62$), in comparison to the DDGS based diets that had a very high correlation coefficient ($r = 0.98$) between observed and simulated values.

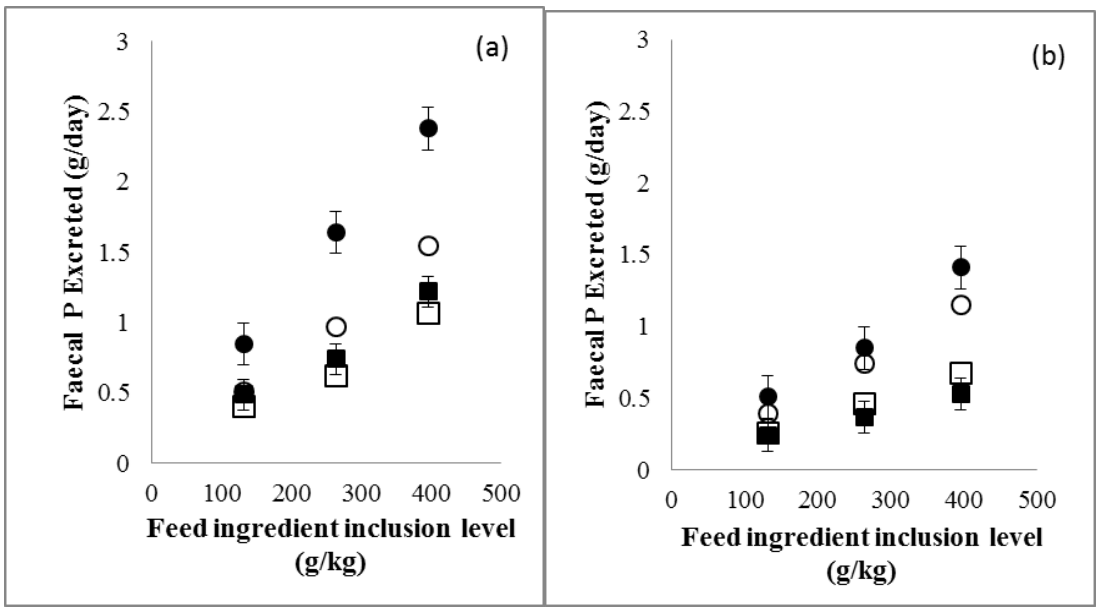


**Figure 3.4** Comparison of experimental observations and their SEM (■,●) by Akinmusire and Adeola (2009) to simulated predictions (□,○) for phosphorus excreted in the faeces. Akinmusire and Adeola (2009) studied the effect of P digestibility in semi-purified diets made of canola (●,○)and soybean meal (■,□) **(a)** without and **(b)** with 1000 FTU of microbial *E. coli* phytase through the gradual supplementation of the feed ingredient investigated.

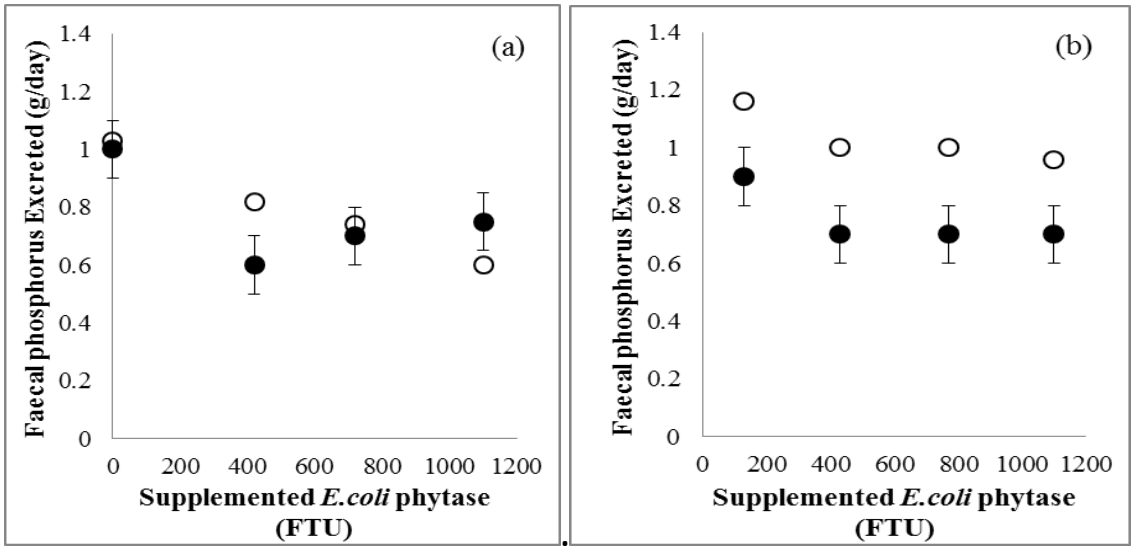


**Figure 3.5** Comparison of experimental observations and their SEM (●) by Almeida and Stein (2012) to simulated predictions (○) for phosphorus excreted in the faeces. Almeida and Stein (2012) studied the effect of graded levels of microbial *E. coli* phytase supplementation for **(a)** corn based diet and **(b)** dried distillers grains with solubles (DDGS) based diets.

Jendza and Adeola (2009) tested the effect of graded levels of microbial phytase enzymes on soluble P excreted. The observed total and soluble P excreted were in good agreement with the predicted results with high correlation coefficients, 1.00 and 0.95, respectively (**Figure 3.6**). The low CV-RMSE of the predicted total and soluble P excreted, indicated the closeness of the predicted to the observed values. In addition, both predicted total and soluble P excreted had low bias values, in comparison to the observed results.

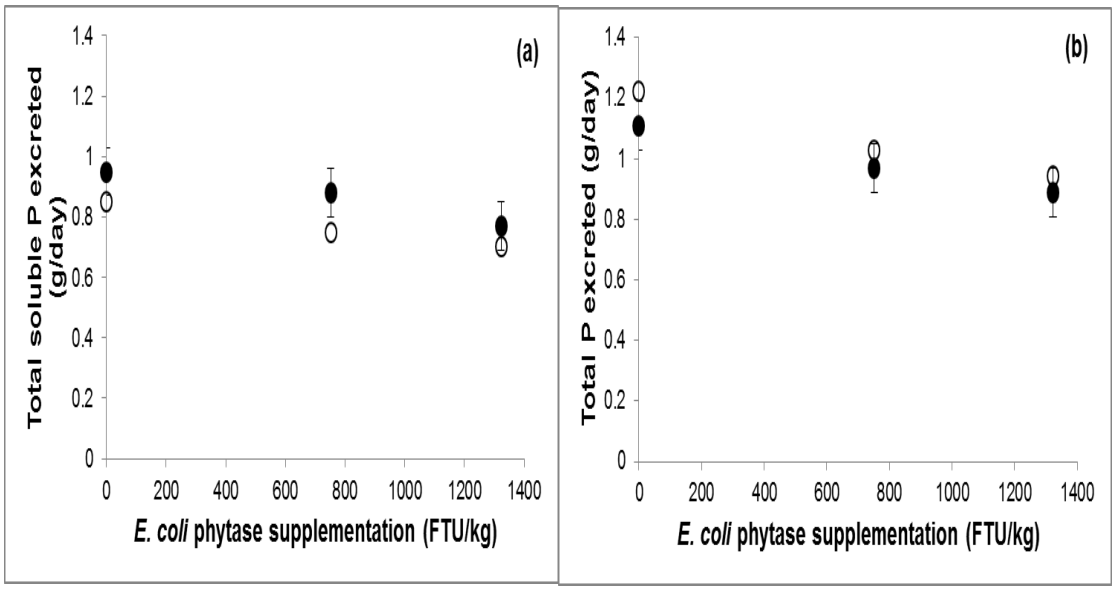


**Figure 3.6** Comparison of experimental observations and their SEM (●) by Jendza and Adeola (2009) to simulated predictions (○) of **(a)** total soluble P excreted and **(b)** total P excreted at graded levels of microbial *E. coli* phytase supplementation.

### *3.4.2.4 The effect of increasing dietary levels of Ca*

Stein et al. (2011) determined the effect of different dietary Ca levels on P retention and excretion in g/day. It can be seen from **Figure 3.7** that the observed and predicted P retained decreased with increasing dietary Ca. The relatively poor agreement in the rate of change in P retained and excreted between observed and predicted was reflected in the low correlation coefficients, of 0.48 and 0.25, respectively, see **Table 3.3**. Despite the predicted measurements not being able to follow the same pattern as the observed results, there were low CV-RMSE and E values, for both retained and excreted P, indicative of the lack of bias and closeness of fit between predicted and observed values.

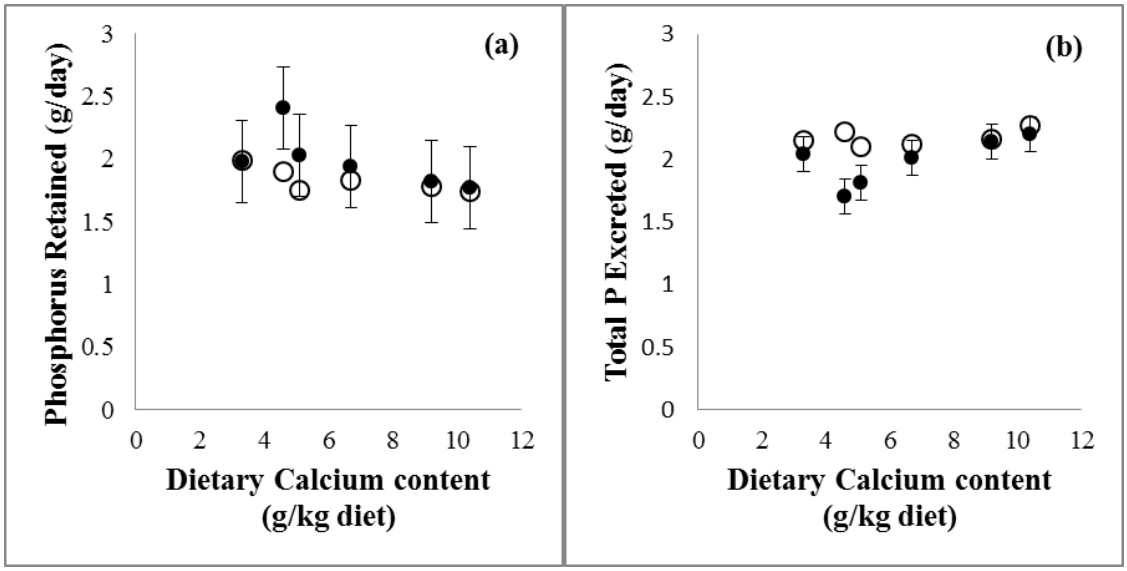


**Figure 3.7** Comparison of experimental observations and their SEM (●) by Stein et al. (2011) to simulated predictions (○) of **(a)** retained P and **(b)** total P excreted at graded levels of dietary Ca.

Finally, Poulsen et al. (2010) evaluated the effect of dietary Ca content with and without microbial *A. niger* phytase supplementation in a diet with high intrinsic phytase, due to the high concentration of wheat in the diet. The simulated and observed data for P retained without phytase supplementation decreased with increasing dietary Ca content (r = 0.42) (**Figure 3.8)**. When 750 FTU phytase was supplemented the observed P retained increased with increasing dietary Ca, but this was not the case for the predicted values (r = -0.75). The predicted P retained without phytase supplementation had 3.75 times lower CV-RMSE than the predicted P retained with phytase supplementation, compared to the observed values. Increasing the dietary Ca content caused an increase in the total P excreted in both observed and predicted values for the unsupplemented diet, with a relatively high correlation coefficient (r = 0.69). The opposite was the case for the phytase supplemented diets as far as total P excreted was concerned (r = -0.96) between observed and predicted results.

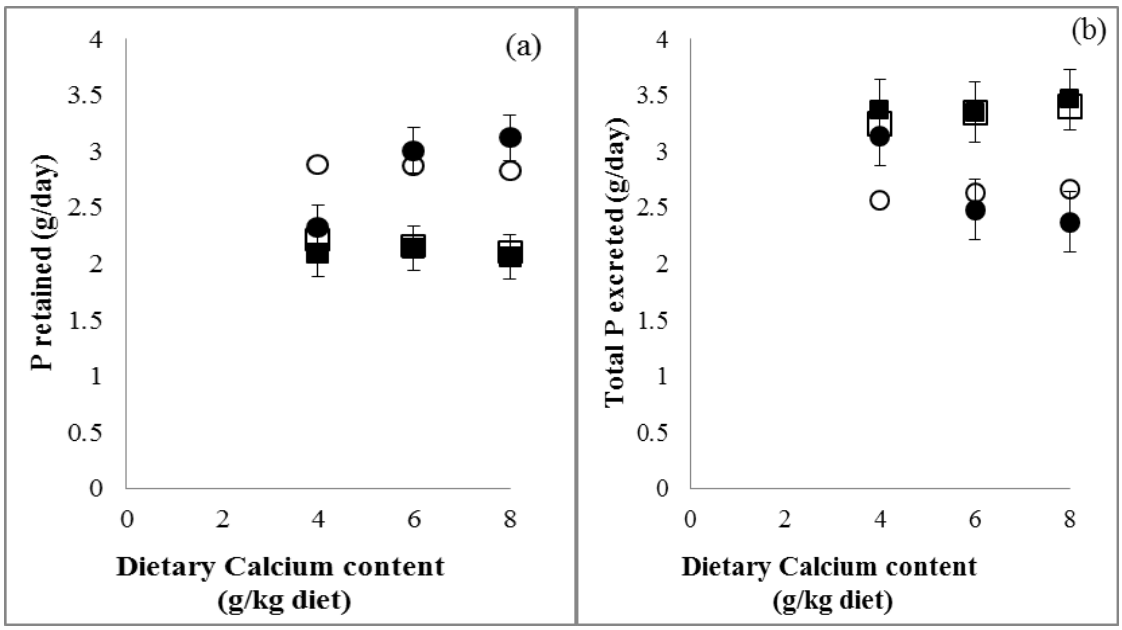


**Figure 3.8** Comparison of experimental observations and their SEM (■,●) by Poulsen et al. (2010) to simulated predictions (□,○) of **(a)** retained P, **(b)** total P excreted. They investigated the effects of different levels of dietary calcium, with (●,○) and without (■,□) 750 FTU of microbial *A. niger* phytase and the diet had FTU/kg diet plant phytase activity.

## 3.5 Discussion

### *3.5.1 Sensitivity analyses*

The sensitivity analysis revealed that only four parameters were able to influence model outcomes to a major extent. Model parameters $e_{growth}$ and $K_{abs.NPP}$ had a big effect on the predictions of P retained, and total and soluble P excreted. The parameter dealing with the maximum oP dephosphorylation by *E.coli* phytase ($K_{max.E.coli}$), influenced only the predictions for P retention and total P excreted, but not soluble P excreted. Finally the maximum oP dephosphorylation by large intestine phytase ($K_{max.LI}$) parameter influenced the predictions only for the different forms of P excreted, i.e. soluble and insoluble.

The $e_{growth}$ parameter determines the amount of digestible P retained in the body. Therefore, it has a direct impact on urinary P excreted and determines requirements. A lower value of $e_{growth}$ would result in a higher excretion of soluble P through the urinary tract, as well as higher requirements for digestible P. We have previously stated that

despite the importance of estimating this parameter accurately, it is very surprising that there is considerable uncertainty over its value and how this is affected by the animal intrinsic factors, such as body weight, sex and genotype. In this model due to lack of existing data on its variation, the value of this parameter was kept constant across these factors, in line with Sandberg et al.(2005).

Decreasing the value of $K_{abs.NPP}$ in the model, resulted in a reduction of phosphate absorbed into the blood-stream; as a result less digestible P was available for retention. The phosphate not absorbed into the bloodstream is excreted through the faeces and as a consequence there is an amount of soluble P being excreted in them (Jendza and Adeola, 2009). Interestingly, when the value of the $K_{abs.NPP}$ parameter was increased the percentage change in the investigated model outputs was smaller than when this parameter decreased. This demonstrates the fact that the model outputs were relatively insensitive to the mechanism of P absorption (i.e. passive or active). The effect of an increase in the parameter is the result of digestible P intake exceeding the maximum P retention. A 20% higher $K_{abs.NPP}$ coefficient meant that there was an increase in the digestible P, which could not be retained and was excreted as soluble P under the default conditions used (Ekpe etal., 2002).

A reason for $K_{max.A.niger}$ not exhibiting a major influence on model predictions, compared to the influence of $K_{max.E.coli}$, is due to the fact that the model calculates that the rates of oP dephosphorylation are different among commercial phytases, see chapter 2. Because more oP is dephosphorylated with *E. coli* compared to *A. niger* at the default phytase activity (750 FTU), the same level of change in the parameters had a greater impact on model outputs when *E. coli*, as opposed to *A.niger* phytase was supplemented.

The final parameter with a major effect in the sensitivity analysis was $K_{max.LI}$, resulting in a 10% change in soluble P excretion. This is because the endogenous microbial large intestine phytase has a major role in oP dephosphorylation (Sandberg et al., 1993; Fan et al., 2001). The dephosphorylated oP in the large intestine does not play an important role in P retention, because the resulting phosphate is not absorbed into the bloodstream from the large intestine (Jongbloed et al., 1992; Peerce, 1997) and is excreted as soluble P (Jendza and Adeola, 2009). Therefore, an accurate quantification of oP dephosphorylation in the large intestine as a function of dietary Ca and other cations, such as Zn, Fe and Mn, and protein (Selle et al., 2011), is crucial in the prediction of the

soluble and insoluble P excreted to the environment. More experiments are needed to provide more confidence in the value of the $K_{max.LI}$ parameter.

### *3.5.2 Comparison of model with published trials*

The model predicted very accurately the direction of response to inorganic P supplementation for digested, retained and total P excreted in the experiments of Ekpe et al. (2002) and Lopes et al. (2009). The agreement with the experiment of Lopes et al. (2009) also gives some confidence in the value of 0.8 for the digestibility coefficient used in the model and on the assumption that in pigs there is little regulation of P absorbed from the lumen into the bloodstream.

The model also accurately predicted P retained and excreted for different dietary phytate levels in the experiment by Trujillo et al. (2010). The model, however, underestimated the retained P and overestimated excreted P at the highest level of phytate, whilst supplemented with phytase. The explanation for this inconsistency may lie in the fact that the first-order kinetics equation used to describe the relationship of oP dephosphorylation with *A. niger* phytase may not have been sufficiently sensitive to describe dephosphorylation at the high concentrations of dietary oP used, which were well beyond the oP concentration of normal, commercial diets.

The model only slightly underestimated the effects of phytase addition to a corn-soya based diet on soluble P excreted (Jendza and Adeola, 2009). Such an under-estimation may imply that there was more phytate dephosphorylation in the large intestine than the model predicted. As the model was developed with the aim of predicting the different forms of P excreted, the relatively good agreement between observed and predicted values of P excreted is important. The major failures of the model were in its inability to predict accurately the P retention and excretion on the canola and DDGS diets supplemented with phytase and, to a lesser extent, on diets with low levels of Ca.

The model significantly under-estimated the faecal P excretion on canola based diets, for the study of Akinmusire and Adeola (2009). This under-estimation by the predictions was the result of over-estimation of the P digested (data not shown). The over-estimation of predicted digested P could be attributed to: (1) lack of phytate

digestion by dephosphorylation from the small intestine phytase; or (2) the digestion coefficient used by the model to simulate the absorption of phosphate from the lumen to the blood-steam was an over-estimate. The model was developed on the basis that no Ca-phosphate complexes would be formed in the small intestine, even though according to Létourneau-Montminy et al. (2011) this is theoretically possible. The justification for this model assumption was the 11 times greater affinity of Ca to phytate, than to phosphate (Luttrell, 1993). In a realistic pig diet, there will always be enough phytate reaching the small intestine to form phytate-Ca in preference over phosphate-Ca complexes.

The differences in the predictions of phytate dephosphorylation in canola- and soyabean meal-based diets (Adeola et al., 2004) may be due to the differences in the storage sites of phytate in the feed ingredients. In soybean seeds, oP is located within protein bodies distributed throughout the cotyledon tissue which constitutes 90% of the seed, while in canola, oP is found in globoids inside protein bodies situated in the radicle and cotyledons comprising about 80% of the seed (Blaabjerg et al. 2010). This has led some to suggest the term ‘reactive’ phytate in order to account for the inability of phytase to dephosphorylate plant phytate (Leske and Coon, 1999). If the ingredients are needed to be classified in terms of the ‘reactive’ and ‘non-reactive’ phytate content, then this would impose additional requirements on the ‘sufficient’ description of the pig diet. Currently, we are not aware of a readily available feed evaluation test that will allow this, although a measurements of the solubility of phytate in pig diets may be one way forward (Létourneau-Montminy etal., 2011). Similarly, the fibre content of the diet may play an important role in this response, although currently we do not have any data available to test this effect.

The over-estimation of the predicted faecal P excretion for a DDGS based diet, from Almeida and Stein (2012), implies that there was an under-estimation of the predicted standardised P digestion, compared to observations. Such an under-estimation of P digestion could be a reflection of the higher amount of P being actually present as digestible myo-inositol dihydrogen phosphate (IP), IP2 to IP5, in the case of DDGS, instead of the assumed, dominant IP6 that characterises the majority of P in plant based feed ingredients (Zijlstra and Beltranena, 2009). The partial break-down of IP6 in DDGS is usually the result of fermentation and drying process, from the production of fuel ethanol.

The model showed a moderate qualitative and quantitative agreement with the measured P retained and total P excreted of the studies of Stein et al. (2011) and Poulsen et al. (2010) who investigated the effects of different levels of limestone supplementation. The predicted and observed P retained in the study of Stein et al. (2011) followed the same pattern when limestone was supplemented, indicating that the model accurately predicted the effect that dietary Ca had on P retained and excreted. There was a linear decrease between limestone supplementation and P retained, attributed to the higher availability of Ca cations, acting as substrate for the formation of indigestible Ca-phytate complexes, therefore limiting the absorption of P into the bloodstream for retention. In this study the P content of the diet was relatively low, explaining the negative effect of increasing Ca on P digestion.

Nevertheless, the model over-estimated the P retained and hence underestimated the P excreted when phytase was supplemented at low dietary Ca content diets in the study of Poulsen et al. (2010). Such an over-estimation would be accounted for by the suggestion of Létourneau-Montminy et al. (2012) who stated that if insufficient amount of dietary Ca is digested then digestible P cannot bind with digestible Ca for bone formation, therefore digestible P is excreted through the urinary tract. The model could not predict Ca digestion in adequate terms, but was rather assumed that there was enough Ca digested for P retention to take place. Thus the model would benefit from a more careful consideration and representation of Ca digestion and the interaction between dietary Ca and the different forms of P in the digestive tract of the pig.

### 3.6 Conclusion

The model satisfactorily predicts the pig responses in terms of P digested, retained and excreted to variation in: (1) inorganic P supply; (2) phytate; and (3) phytase. However, the model predicts less accurately the P retention and ultimately P excretion using unconventional diets, such as diets containing a significant proportion of canola meal and DDGS. Although the model is able to predict the effect of Ca supplementation on P digestion in Ca-abundant diets, there are inconsistencies in model predictions that may arise from the interactions between Ca and the different forms of P in the digestive tract. Currently this is an area of research where effort is being directed (Selle et al., 2011). In

conclusion, given its relative success in accurately predicting pig responses to dietary variations in P content, the model may be applied to develop feeding strategies to optimise P retention and minimize P excretion, therefore, decreasing the feed costs and the environmental impacts in growing and finishing pig operations.

### 3.7 Implications

The model developed in the previous chapter paper and evaluated here predicts adequately the P digestion, retention and excretion of growing-finishing pigs for a wide range of dietary compositions and for pigs of different genotypes. Consequently, the model can be applied to develop feeding strategies to optimize P utilization and minimize the different forms of P excreted to the environment. The model can be further improved, by considering 'reactive' as opposed to total phytate content of the diet, as well as experimentally establishing the net efficiency of digestible P utilization for growth and the non-phytate P absorption coefficient from the small intestine into the bloodstream, for pigs offered access to different diets.

# Chapter 4. Quantifying the consequences of nutritional strategies aimed at decreasing phosphorus excretion from pig populations

## 4.1 Abstract

There is a global imperative to reduce phosphorous (**P**) excretion from pig systems. Here, we modified a deterministic model that predicts P digestion, retention and metabolism for different pig genotypes given access to different feeds, into a stochastic one to investigate the consequences of different management strategies on P excretion by a group of pigs growing from 30 to 120 BW. The conversion was associated with several challenges, including the description of the variation and co-variation between the different parameters that describe pig genotype. The strategies investigated were: (1) changing feed composition frequently in order to match more closely pig digestible P (**digP**) requirements to feed composition (phase feeding); and (2) grouping pigs into a light and a heavy group and feeding each group according to the requirements of their group average BW (sorting). Phase feeding reduced P excretion as the number of the feeding phases increased. The effect was more pronounced as the feeding phases increased from 1 to 2, with a 7.5% decrease achieved; the increase of the phases from 2 to 3 was associated with a further 2.0% reduction. Similarly, the effect was more pronounced when the feed targeted the population requirements for digP at the average BW of the first third, rather than the average requirements at the mid-point BWof each feeding sequence plan. Increasing the number of feeding phases increased the % of pigs that met their digP requirements during the early stages of growth (30 to 60kg BW) and reduced the % of pigs that were supplied less than 85% of their digP requirements at any stage of their growth. Sorting pigs reduced P excretion to a much lesser extent; the reduction was greater as the % of pigs in the light group increased from 10 to 30% (1.5 and 3.0% reduction, respectively). This resulted from an increase in the P excreted by the light group and a decrease in the P excreted by the remaining pigs. Sorting increased the % of light pigs that met their dig P requirements and only slightly decreased the % of remaining pigs that met these requirements at any point of their growth. Exactly the converse was the case as far as the % of pigs that were supplied less than 85% of their digP requirements were concerned. The developed model is flexible and can be used to investigate the effectiveness of other management strategies to reduce P excretion from groups of pigs, including precision livestock feeding.

## 4.2 Introduction

As well as phosphorus (**P**) being the most expensive feed resource after energy and protein, its excretion is an important aspect of the environmental impact of livestock systems. The water soluble P excretion represents the highest potential risk for losses by runoff in agricultural fields, causing eutrophication (Maguire et al., 2005). It has been estimated that pigs contribute ~15% of the total diffuse P load from livestock to waters in Great Britain (White and Hammond, 2006); in N. America the nutrient, including P, content of manure and its impact on the environment is considered a major challenge for pig systems (Statistics Canada, 2006). It is therefore an imperative to develop strategies that minimize P excretion from pig systems.

Although there is some potential to reduce P excretion by genetic means (Forsberg et al., 2003), reducing P excretion by nutritional and management means remains the most viable option (Kyriazakis et al., 2013). In this paper we quantified the consequences of different nutritional management strategies on P excretion by groups of pigs through simulation modeling. The strategies investigated were: (1) changing feed composition frequently in order to match more closely pig requirements to feed composition (phase feeding); and (2) grouping pigs and feeding them according to their group average BW (sorting).

The investigation required a stochastic methodology, to take into account the variation between individual pigs and its effect on groupP retention and excretion. Currently there are a limited number of stochastic models that may enable us to address questions about nutrient excretion from pigs systems (Ferguson et al., 1997; Knap, 2000; Schinckel et al., 2007; Brossard et al., 2009). Of those only Pomar et al. (2009; 2011) is capable of dealing with P and has addressed the consequences of phase feeding on total P excretion. There are no stochastic approaches that enable the prediction of P excretion in soluble and insoluble forms.

## 4.3 Material and methods

### *4.3.1 Single animal model description*

The dynamic, deterministic pig growth model of Wellock et al (2003), as adopted in chapter 2 was used to predict the fate of dietary P in groups of pigs. The model operated in daily time steps, and considered pigs maintained in a thermo-neutral environment, growing from 30 kg BW until they reached a UK slaughter weight of 120 kg BW. No environmental stressors were assumed to operate on the pigs (Wellock et al., 2004). The main model inputs were: (1) pig genotype, including initial state; (2) food composition; and (3) feeding plan; while the model outputs for an individual pig were: (1) average daily gain; (2) body composition; (3) food intake and (4) soluble and insoluble, and hence total P excreted.

The initial state of the pig was described by its initial body weight (**BW0**), from which the chemical composition of the pig was calculated assuming that the pig had its ideal composition setby its genotype (Emmans and Kyriazakis, 2001). The potential rate of protein retention was determined by pig genotype and current protein weight only. The maximum (potential) protein retention was then used to determine the potential gains of the other chemical components, including P (Emmans and Kyriazakis, 1997; Wellock et al., 2003). Potential average daily gain was the sum ofthe potential gains of protein, lipid, ash (including P) and water. Five percent of the BW gain was assumed to be gut fill (Wellock et al., 2004).

Each pig was given access to a feed of a certain P content (see below). It was assumed that the pig will attempt to consume an amount of feed that will satisfy its energy and protein requirements for potential daily gain and maintenance (Kyriazakis et al., 1990). The same regulation does not seem to apply for P (Pomar et al., 2006; Lopes et al., 2009). The amount of feed that allows the pig to meet its and protein requirements to be achieved was calculated from the current protein and lipid contents of the pig, and the composition of the feed. If the feed was deficient in P then the actual, as opposed to potential rates of retention were calculated. In chapter 2 we predicted the P digestion,

retention and ultimately excretion in growing and finishing pigs of different genotypes, offered access to feeds of different P content. The total P excreted comprised of fecal and urine P. The feces contained both insoluble and soluble P, while urinary P was only soluble (Jendza and Adeola, 2009; Selle et al., 2011). For a complete description of the model including inputs and outputs, see chapter 2.

### *4.3.2 Generating variation in pig genotype*

The pig genotype was described by the following model variables: protein at maturity (**$Pr_m$**, kg), lipid to protein ratio at maturity (**$LPr_m$**, kg/kg) and growth rate (**B,** per day), in accordance with Ferguson et al (1997), Knap (2000), Emmans and Kyriazakis (2001), Pomar et al (2003) and Wellock et al (2004). The scaled rate parameter, $B^* = B.\ Pr_m^{0.27}$, described by Emmans and Fisher (1986), was used as an alternative to B to avoid the problems caused by correlations between B and $Pr_m$. The values of B*, $Pr_m$, and $LPr_m$ were assumed to be uncorrelated and normally distributed (Ferguson et al., 1997; Knap 2000; Pomar et al., 2003; Wellock et al., 2004).

The mean and SD of $Pr_m$was estimated from the study of Knap et al (2003) to be 35 and 4.38 kg, respectively. The mean and SD of B* was estimated at 0.0392 and 0.0078 kg/day, respectively, calculated from Brossard et al. (2009), who in turn derived it from the data of Rivest (2004). Finally the mean and SD of $LPr_m$ were derived from Knap and Rauw (2008) to be 1.50 kg/kg and 0.315 kg/kg, which were in turn adapted from Doeschl-Wilson et al (2005). The mean $Pr_m$ was 9% higher, while the B* and $LPr_m$ were 4 and 8% lower, respectively, from those proposed by Wellock et al. (2004) which were based on the genetic line of van Lunen (1994). The changes in these values are consistent with genetic changes that have taken place in pig genotypes over a period of 10 years.

The model concentrated on variation in the genetic parameters, B*, $Pr_m$ and $LPr_m$. By varying the values of these parameters, it was possible to use the model to describe the actual genetic variation in pig performance, including both growth and maintenance

requirements. The model assumed a constant digestive coefficient for P and a constant net efficiency digested P utilization, in accordance with Kyriazakis (2011). Even under the best growing conditions, there is likely to be variation in initial state between pigs at the start of a growing period (Wellock et al., 2004). Individual variation in BW0 was generated from the assigned genotype mean (**μBW0**,kg) and standard deviation (**σBW0**,kg) of BW0 using the simulated genetic parameters of the individual to correlate BW0 with potential growth. This implied that individuals with the highest genetic potential within the group tended to have the highest BW0 and as a result continued to grow faster and reached slaughter weight earlier than their counterparts, and therefore had different digestible P (**digP**) requirements at each stage of growth. The initial BW for individual pigs (**BW0*i***) was calculated in accordance with Wellock et al. (2004), while the initial chemical composition of each pig was calculated from BW0*i*. The σBW0 is the SD BW of the population, derived from Wellock et al. (2003) was 3.35 kg. The model was expected to generate a population with a coefficient of variation **(CV)** of 10% and 15% at 30 kg and 90 kg BW, respectively (Wellock et al., 2003).

A stochastic Monte-Carlo simulation was used, created inVisual Basic Application (**VBA**) in Microsoft Excel 2010, to simulate 500 individual pigs that composed a population, equivalent to a batch going through a UK farm at a particular point in time. For each simulated pig within the population, values for B*$i$ , $Pr_m i$, and $LPr_m i$ were drawn at random from uncorrelated normal distributions for each of the genetic parameter using their mean and SD values. Therefore, 500 individual B*$i$ , $Pr_m i$, and $LPr_m i$ were drawn at random and were used to generate BW0*i*.

In Monte Carlo simulations, the number of simulations used is a compromise between the accuracy of the output (e.g. the estimate of the mean value) and the requirements of computing power. As the standard error of the output is directly dependent on the size of the sample, increasing the number of model runs will automatically improve the accuracy. However, in practice, Monte Carlo runs, especially with a complex simulation model, are time consuming, and this often determines the upper limit for the simulations to be used. In this study 500 runs (500 individuals) were sufficient because the standard errors for the predicted mean values were less than 0.5%.

### *4.3.3 Feeding strategies*

#### *4.3.3.1 Phase feeding*

Three feed sequence plans were investigated; feeding one, two or three different digP diets over the course of 30 to 120 kg average BW, for the population of 500 pigs, with varied genetic parameters and BW0. Feeds in all simulations were identical in net energy (9.68 MJ/kg), crude protein (17.25%) and Lysine (1.11%). The pigs were offered *ad libitum* access to the diet. No feed resource other than P could limit growth in the simulations. In this respect, the above methodology was in accordance to Brossard et al. (2009), who investigated the variation of the population performance in response to lysine, rather than P.

The simulated base-line diet, currently in use by the UK pig industry, had a chemical composition of 5.19 g total Ca and 4.29 g total P/kg. The dietary total P consisted of 2.47 g phytate (**oP**) and 1.82 g phosphate P (**NPP**) /kg feed, and total digP was 2.67 g/kg. The average daily digP requirements (g/kg feed) of the population were responsible for the changes seen in **Table 4.1** in the digP and total Ca content of the diet (g/kg feed) used. Within each phase of a feed sequence plan, the digP requirements (as g/kg feed) of the population declined and so did the digP supplied. The feed changed when the average BW of the population reached the end of each phase (sequence plan). When the digP feeding regime changed, the oP:NPP and Ca:digP ratios also changed (**Table 4.1**). The dietary exogenous phytase supplementation (*E. coli*) was constant through-out all phase feeding strategies, at 750 FTU/kg.

**Table 4.1** The digestible P (g/kg) contents of the feeds offered to the pigs during each of the feeding phases of a feeding sequence plan: one, two or three phases over the BW range 30 to 120 kg. The supply of dietary digestible P targeted the requirements of the average of the population at the mid-point BW, or the mean BW during the first third of each feeding sequence plan.

| | BW Target (kg) | | Digestible P (g/kg feed) | |
|---|---|---|---|---|
| Feed sequence plan BW (kg) | Half-way target | First-third target | Half-way target | First-third target |
| One phase | | | | |
| 30-120[1] | 75 | 60 | 2.28 | 2.50 |
| Two Phases | | | | |
| 30-74[1] | 52 | 45 | 2.62 | 2.76 |
| 75-120[2] | 97.5 | 90 | 2.02 | 2.10 |
| Three Phases | | | | |
| 30-60[1] | 45 | 40 | 2.76 | 2.84 |
| 61-90[3] | 75 | 70 | 2.28 | 2.34 |
| 91-120[2] | 105 | 100 | 1.94 | 2.00 |

[1]The oP:NPP and Ca:dP ratios used were 1.35:1 and 1.92:1, respectively, and derived from a typical 'grower' UK commercial diet.

[2]The oP:NPP and Ca:dP ratios used were 1.52:1 and 2.50:1, respectively, and derived from a typical 'finisher' UK commercial diet.

[3]The oP:NPP and Ca:dP ratios used were 1.45:1 and 2.21:1, respectively, the intermidiate between the grower and finisher diets.

The changes in the digP and total Ca content of the feed were achieved by changing the amount of supplemented inorganic P and supplemented limestone, respectively. When digP had to be substantially reduced, i.e. in the last feed sequence of the two and three feeding phases, the removal of inorganic P was not sufficient to decrease the digP content of the feed to the levels shown in, **Table 4.1**. It was therefore necessary to decrease a feed ingredient which had high levels of P, and substitute the feed with another ingredient to keep the energy and amino acid content of the feed constant, for all simulations.

The stochastic model determined the daily digP requirements for each individual in the population, based on their genotype, which were then averaged. The study examined the effect of supplying dietary digP to meet the digP requirements of the average of the population at either the mid-point BW (1/2 target) or the average BW of the first third of each feeding sequence (1/3 target; **Table 4.1**). The 1/2 target strategy is often practiced by the industry, whereas the 1/3 target strategy is also practiced but to a lesser extent (Simpson and de Lange, 2012). As the number of phases increases the differences between the digP supplied by the 1/2 and 1/3 target plan diminishes.

#### *4.3.3.2 Sorting according to body weight*

The effect of sorting the lightest 10, 20 and 30 percent BW of a 500 pig population and feeding them a separate digP content feed from the rest of the population on P excreted was investigated. The sorting of the population took place by arranging all pigs in the population, from the lightest to the heaviest, in accordance to the $BW0_i$, at an average 30 kg BW. The sorted and 'rest' population were fed different feeds in terms of digP and total Ca during the BW intervals of 30 to 74 and 75 to 120 kg. The lightest 10, 20 and 30 percent BW had an extra feed sequence plan, until this group reached the average 30 kg BW (**Table 4.2**). Therefore, the sorted pigs were effectively offered three feeding phases, while the 'rest' had two feeding phases. There was also a control simulation, in which no sorting of the population took place.

**Table 4.2** The digestible P (g/kg) contents of the diets offered to the pigs during each of the feeding phases of a 'sorting plan': the pigs were either treated as a single population (no sorting), or the lightest 10, 20 and 30% of the population were fed on a higher digestible P in comparison to the remaining population. The supply of dietary digestible P (g/kg) was determined in order to meet the average digestible P requirements of the sorted and remaining population at the mid-point BW of each feeding phase.

| | Digestible P(g/kg feed) | | |
|---|---|---|---|
| | Feed sequence plan BW/kg | | |
| Sorting Plan | <30[3] | 30 – 74[1] | 75 – 120[2] |
| No sorting | - | 2.62 | 2.02 |
| 10% sorting | | | |
| 10% lightest | 2.99 | 2.77 | 2.12 |
| Remaining population | - | 2.60 | 2.00 |
| 20% sorting | | | |
| 20% lightest | 2.99 | 2.73 | 2.11 |
| Remaining population | - | 2.57 | 1.98 |
| 30% sorting | | | |
| 30% lightest | 2.98 | 2.71 | 2.09 |
| Remaining population | - | 2.56 | 1.98 |

[1]The oP:NPP and Ca:dP ratios used were 1.35:1 and 1.92:1, respectively, and derived from a typical 'grower' UK commercial diet.

[2]The oP:NPP and Ca:dP ratios used were 1.52:1 and 2.50:1, respectively, and derived from a typical 'finisher' UK commercial diet.

[3]The oP:NPP and Ca:dP ratios used were 0.61:1 and 1.80:1, respectively, and derived from a typical 'weaner' UK commercial diet.

For each group of pigs, the dietary digP supplied (g/kg diet) met the average digP requirements at half way of each phase (half-way target), i.e. 52 and 97.5 kg BW for the grower (30 to 74 kg BW) and finisher (75 to 120 kg BW) stages, respectively. The sorted pigs were fed a higher digP compared to the 'rest' of the population in order to meet their higher digP requirements (**Table 4.2**). The time taken for each sub-population to reach the target BW was recorded. The baseline feed fed to each group was the same with the phase feeding regime, having the same composition and nutritional value, with the only exception being its P and Ca level (see above). The higher digP requirements of the pigs less than 30 kg BW required the supplementation of the feed with mono calcium-phosphate and limestone to achieve the digP and total Ca contents (**Table 4.2**). The rules used for the change in the digP and Ca contents of the feeds offered to the remaining of the population were the same as for phase feeding.

### *4.3.4 Simulation outputs*

From the generated simulated populations, which were fed according to the strategies described above, the following outputs were calculated: (1) the cumulative P excretion as total, soluble and insoluble P (kg); (2) the population performance (mean and CV) in terms of BW gain (kg/d), Pr and P retained (g/d) and food conversion ratio; (3) the percentage of the population that had their digP requirements met throughout the BW period 30 to 120 kg of the population; and (4) the percentage of population that were supplied less than 85% of their requirements at any one stage of their growth, in order to identify the level of P underfeeding that happened within the population.

The cumulative soluble and insoluble P excretion for each pig was calculated by adding the daily soluble and insoluble P excreted, respectively, to derive the total amount of soluble and insoluble P excreted to the environment from 30 to 120 kg BW for each pig, and subsequently added to calculate the soluble and insoluble P excreted for the whole population of 500 pigs.

Calculating the percentage of the population that had their digP requirements met, required the comparison of the daily digP intake (g/d) against the daily digP requirements (g/d), for each pig for each day. If the daily digP requirements were

greater than the daily digPinput, this was recorded as a 'No'; otherwise it was recorded as a 'Yes'. The number of 'Yes' signs, in the population per day was quantified, in order to identify the percentage of pigs that had their requirements met at each day.

In order to quantify the percentage of population that were supplied less than 85% of their requirements, it was first necessary to identify the level of underfeeding or overfeeding of digP for each pig for each day, compared to its daily requirements. These data were used to count the number of pigs that were supplied less than 85% of their requirements for each day in a population. Calculating the percentage population supplied with less than 85% of their requirements was in accordance with NRC (2012), which states that if pigs are undersupplied with digPby more than 15% of their requirements, this will negatively affect their growth.

## 4.4 Results

### *4.4.1 Phase feeding*

As the number of feed phases increased over the BW period 30 to 120 kg, the amount of cumulative P excreted by the population of pigs decreased (**Table 4.3**). There was an average decrease of 7.50 and 9.29% in total cumulative P excreted, when the feeding phases increased from one to two and from one to three, respectively. Similarly the largest decrease in soluble and insoluble cumulative P excreted was seen when the feeding phases increased from one to two. The cumulative P excreted was lower when the 1/2 target, as opposed to the 1/3 target was used; this was consistent across all feed sequence plans. When the 1/2 target feeding regime was used, 13.9, 8.24 and 3.84% less soluble P was excreted, in comparison to the 1/3 target feeding regime, for each of the phase feeding sequences (1, 2 and 3 phase feeding, respectively). Across all phase feeding plans used, soluble P contributed ~75% of the total P excreted. The standard errors of the estimated mean values for the total P excreted were relative low ~1% for all phase feeding scenarios, which indicates that these estimates reliably represent the true means of the population.

**Table 4.3** The effect of phase feeding (one, two or three phases), on the cumulative total, soluble and insoluble P excreted (kg) from 30 to 120 kg average BW, for a population of 500 pigs, when the supply of dietary digestible P targeted the digestible P requirements of the average of the population at the mid-point BW, or the mean BW of the first third of each feeding sequence plan.

| Phase Feeding | Cumulative P excreted (kg) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Total | | | Insoluble | | | Soluble | | |
| | Half-way change | First-third change | Mean | Half-way change | First-third change | Mean | Half-way change | First-third change | Mean |
| One | 261 | 298 | 280 | 69.1 | 75.6 | 72.4 | 192 | 223 | 207 |
| Two | 250 | 268 | 259 | 67.7 | 70.9 | 69.3 | 182 | 197 | 190 |
| Three | 249 | 259 | 254 | 67.3 | 69.9 | 68.6 | 182 | 189 | 186 |

Increasing the number of feeding phases resulted in a higher percentage of the population meeting their digP requirements during the average BW period 30 to 60 kg (**Figure 4.1 – 4.3**). The converse was the case during the finishing stage of 90 to 120 kg where a lower percentage of population met their P requirements when the feeding phases increased. The use of the one phase feeding resulted in the highest percentage of the population being undersupplied with digP (**Figure 4.2 – 4.6**). Similarly the use of the 1/2 target feeding regime resulted in a higher percentage of pigs being undersupplied, rather than when the 1/3 target feeding regime was used. The majority of the population (> 50%) were supplied less than 85% of their digP requirements from 30 to 48 kg and 30 to 36 kg average BW of the population, through the use of 1/2 target and 1/3 target feeding regime respectively, when the one phase feeding was used. When feeding a two and three phase, the percentage of the population that was underfed never exceeded 50% at any stage of the population growth (maximum of P underfed pigs was 27 and 17%, respectively when thetwo and three phase feeding plans were used).

There was an increase in ADG, Pr and P retained (g/d), and a decrease in the food conversion ratio (**FCR**) when the number of feeding phases increased (**Table 4.4**). In addition, the CV decreased with increasing the number of phases for all the above performance variables. Pigs on the 1/3 target performed better than on the 1/2 target for all investigated performance variables, irrespective of the number of feeding phases. The greatest difference in ADG between the 1/3 and 1/2 target feeding regime, was 0.60% during one phase feeding. In addition, there was a lower CV for the population performance variables, when the 1/3 target was used as opposed to the 1/2 target. Nevertheless, the difference in the population performance between the 1/2 and 1/3 target decreased whilst the number of the feed phases increased.

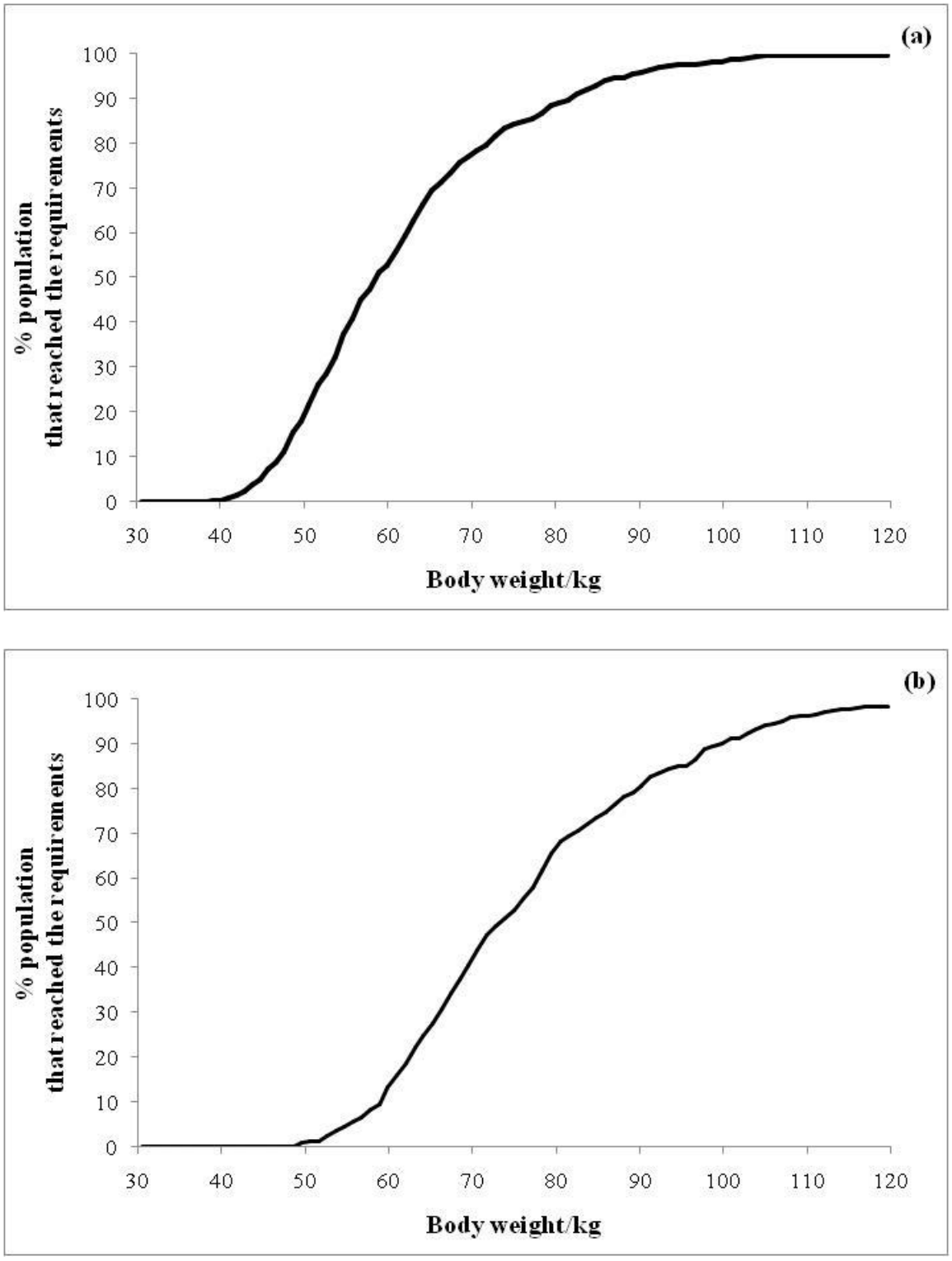


**Figure 4.1** The percentage of the population that met their digestible P requirements over the average BW range 30 to 120 kg, during a one phase feeding sequence plan. The supply of dietary digestible P targeted the digestible P requirements of the average of the population at: **(a)** 60 kg mean BW (first third) or **(b)** 75 kg mean BW (mid-point BW).

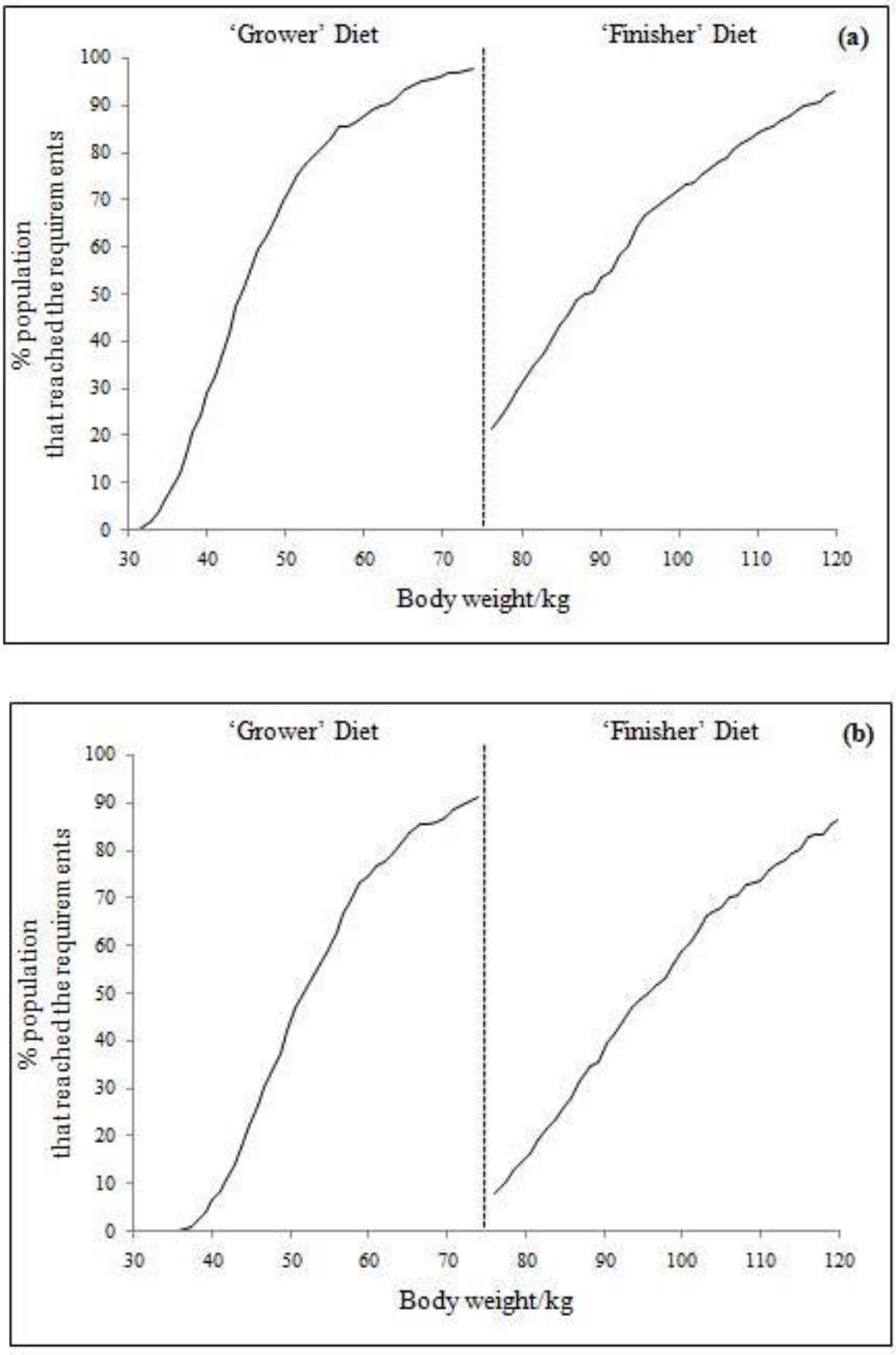


**Figure 4.2** The percentage of the population that met their digestible P requirements over the average BW range 30 to 120 kg, during a two phase feeding sequence plan. The supply of dietary digestible P targeted the digestible P requirements of the average of the population at: **(a)** the mean BW of the first third or **(b)** the mid-point BW of each feeding sequence plan.

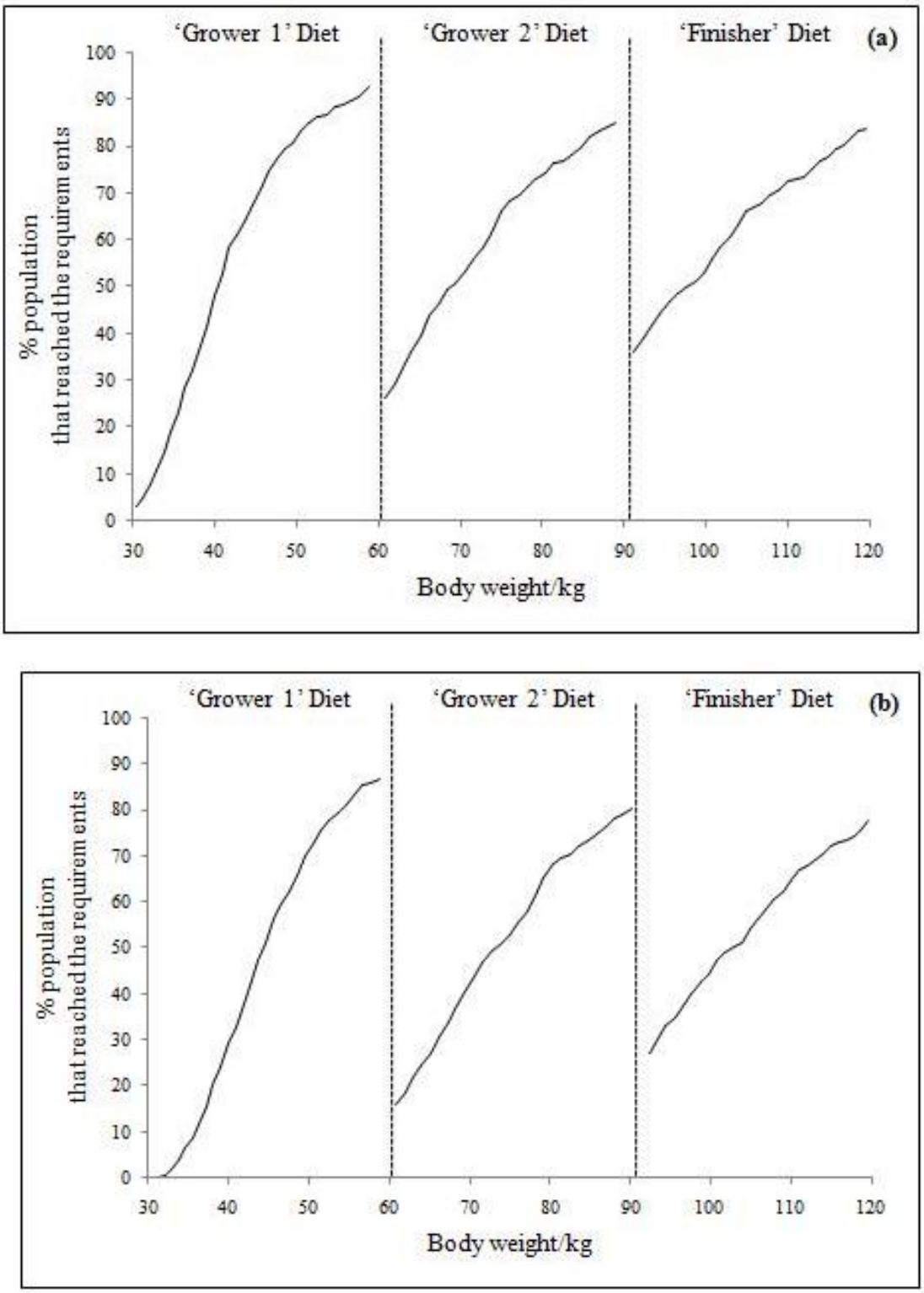


**Figure 4.3** The percentage of the population that met their digestible P requirements over the average BW range 30 to 120 kg, during a three phase feeding sequence plan. The supply of dietary digestible P targeted the digestible P requirements of the average of the population at: **(a)** the mean BW of the first third or **(b)** the mid-point BW of each feeding sequence plan.

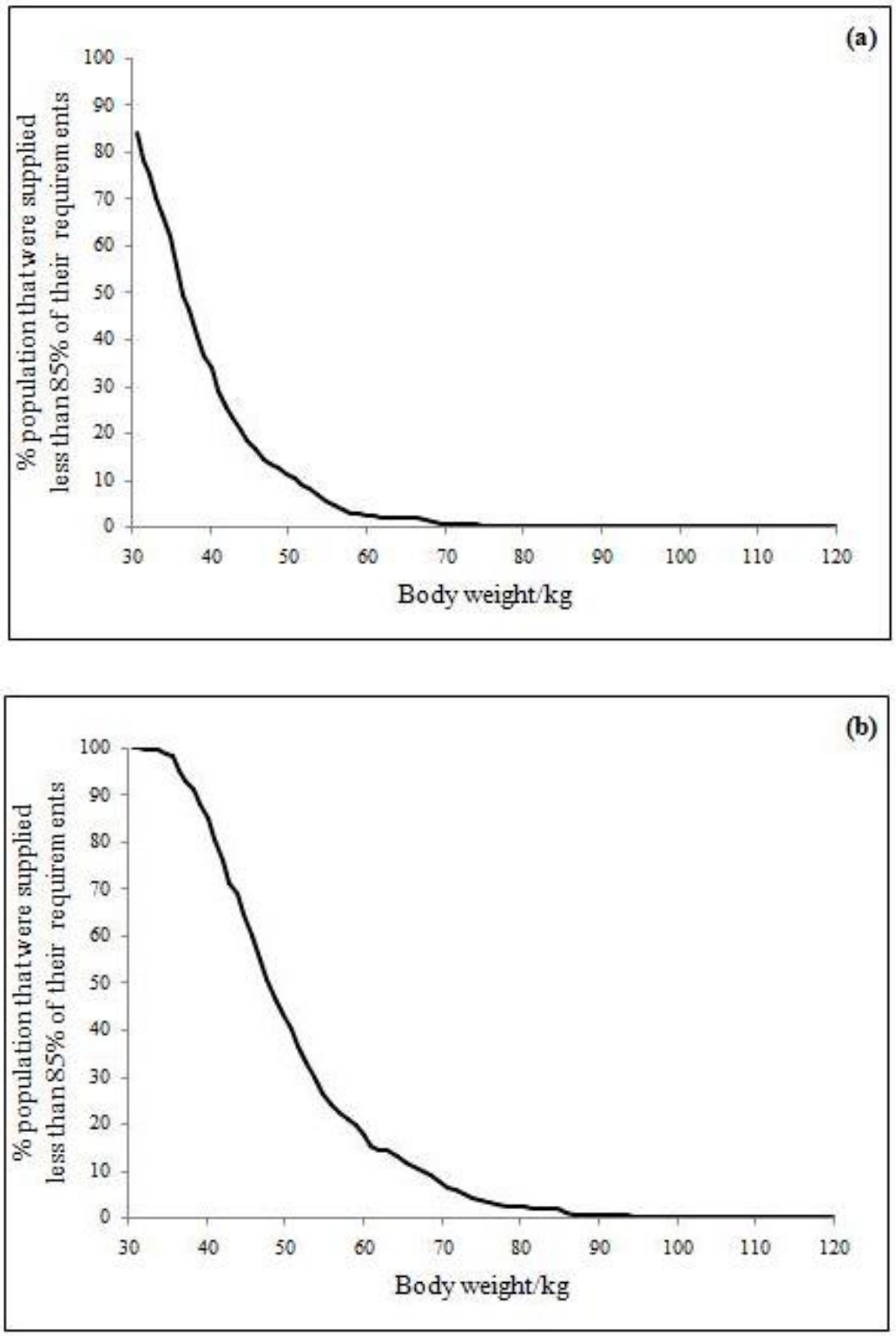


**Figure 4.4** The percentage of population that were supplied less than 85% of their digestible P requirements over the average BW range 30 to 120 kg, during a one phase feeding sequence plan. The supply of dietary digestible P targeted the digestible P requirements of the average of the population at: **(a)** 60 kg mean BW (first third) or **(b)** 75 kg mean BW (mid-point BW).

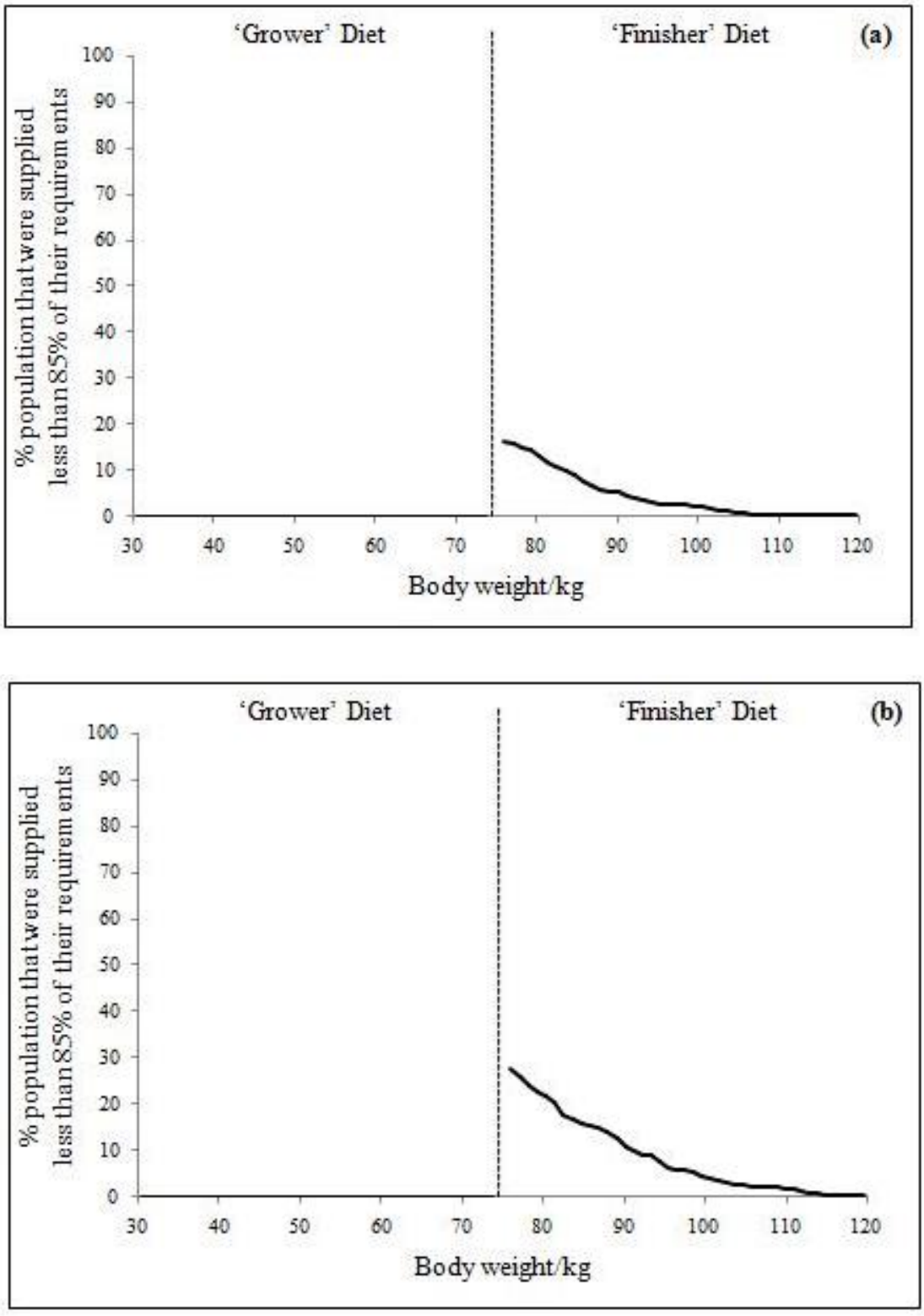


**Figure 4.5** The percentage of population that were supplied less than 85% of their digestible P requirements over the average BW range 30 to 120 kg, during a two phase feeding sequence plan. The supply of dietary digestible P targeted the digestible P requirements of the average of the population at: **(a)** the mean BW of the first third or **(b)** the mid-point BW of each feeding sequence plan.

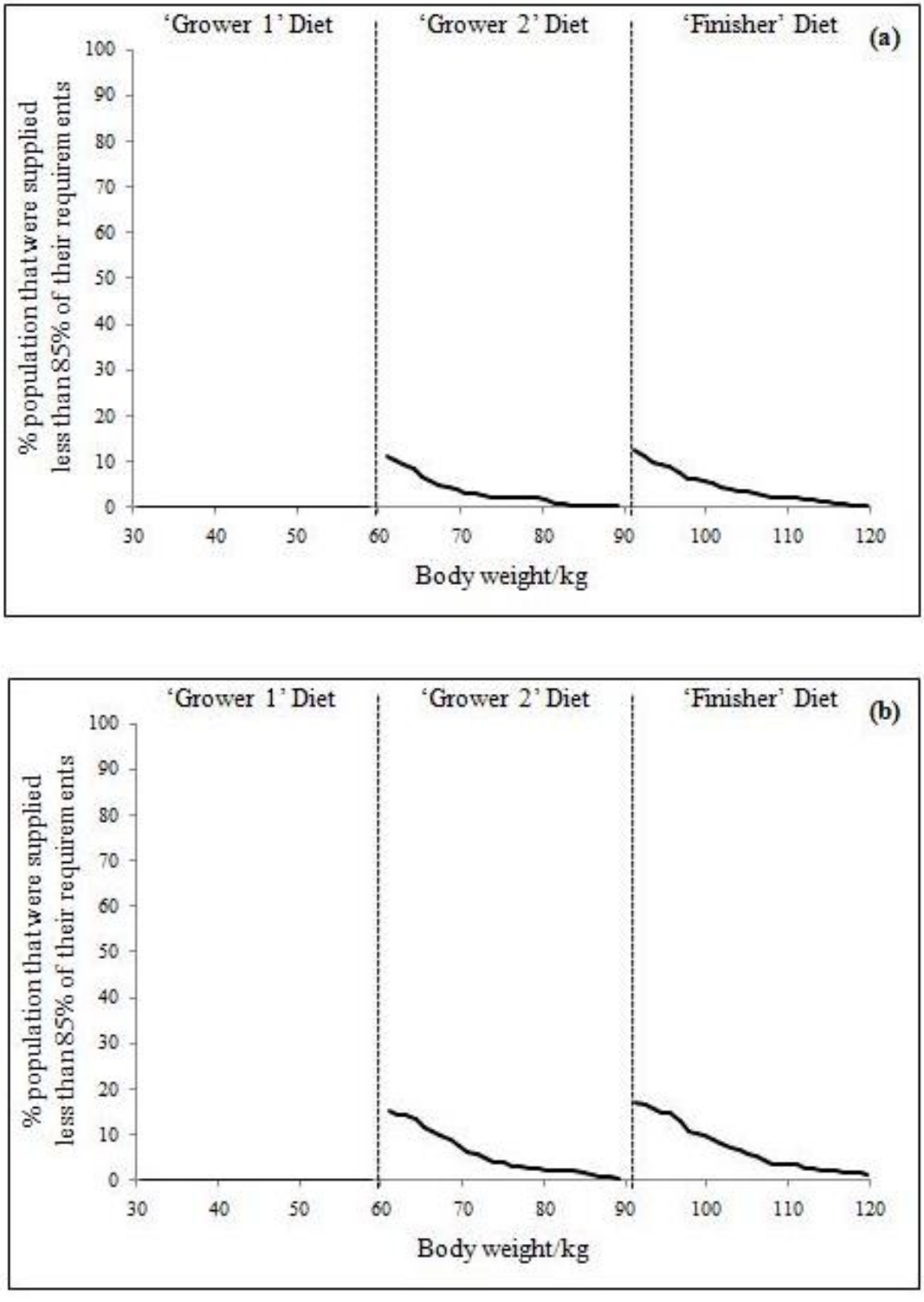


**Figure 4.6** The percentage of population that were supplied less than 85% of their digestible P requirements over the average BW range 30 to 120 kg, during a three phase feeding sequence plan. The supply of dietary digestible P targeted the digestible P requirements of the average of the population at: **(a)** the mean BW of the first third or **(b)** the mid-point BW of each feeding sequence plan.

**Table 4.4** The effect of one, two and three phase feeding on the performance of a population of pigs from 30 to 120 kg in terms of: 1) ADG gain (kg/d); 2) protein (Pr) retained (g/d); 3) P retained (g/d), and 4) food conversion ratio. The supply of dietary digestible P targeted the digestible P requirements of the average of the population at the mid-point BW, or the mean BW of the first third of each feeding sequence plan.

| Feed Sequence plan | BW Target (kg) | ADG (kg/d) | | Pr retained (g/d) | | P retained (g/d) | | Food conversion ratio | |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean | CV | Mean | CV | Mean | CV | Mean | CV |
| 1 | Half-way | 1.006 | 0.1153 | 173 | 0.1345 | 5.44 | 0.1479 | 3.02 | 0.177 |
| | First-third way | 1.012 | 0.0974 | 175 | 0.1041 | 5.64 | 0.1260 | 3.00 | 0.150 |
| 2 | Half-way | 1.024 | 0.0978 | 177 | 0.1005 | 5.60 | 0.1047 | 2.97 | 0.150 |
| | First-third way | 1.025 | 0.0911 | 180 | 0.0926 | 5.72 | 0.0874 | 2.96 | 0.140 |
| 3 | Half-way | 1.027 | 0.0929 | 180 | 0.0960 | 5.65 | 0.0909 | 2.96 | 0.143 |
| | First-third way | 1.029 | 0.0901 | 182 | 0.0895 | 5.75 | 0.0809 | 2.95 | 0.140 |

### *4.4.2 Sorting according to body weight*

Sorting pigs into 'light' and 'remaining' groups, increasing the size of the light group and feeding each group in accordance to their average digP requirements resulted in a decrease in the cumulative P excreted by the population as a whole (**Table 4.5**). There was a 1.32, 1.92 and 3.04% reduction in the cumulative total P excreted by the population as a whole, when 10, 20 and 30% of the population were sorted, in comparison to the equivalent group in the population that was not sorted. The cumulative total P excreted by the sorted lightest 10, 20 and 30% of the population increased by 49, 43 and 40%, respectively, compared to the equivalent group of the population when not sorted. The converse was the case for the remaining of the population, as 'remaining' pig excreted 5.17, 9.91 and 16.2% less total P, respectively, compared to the equivalent group of the population that was not sorted. Across all sorting regimes used, soluble P contributed ~75% of the total P excreted. The standard errors of the estimated mean values for the total P excreted were relatively low ~1% for all sorting scenarios, which indicates that these estimates reliably represent the true means of the population.

As expected a larger percentage of the 'light' pigs met their P requirements at any stage of their growth compared to the equivalent group of the population that were not sorted (**Figure 4.7 – 4.9**). The largest difference between sorted and not sorted light pigs in the percentage of pigs that met their requirements, was between 60 to 75 kg BW. The 'remaining' population had a much smaller difference between sorted and not sorted pigs in the percentage of pigs that met their requirements, in comparison to the 'light' group. The percentage of population that met their individual digP requirements was increasing with increasing BW of the average population. The only exception to this trend was at the initial stages of growth for the 'light' group, which was relatively constant.

**Table 4.5** The total, soluble, and insoluble cumulative P excreted by a population of 500 pigs treated according to a 'sorting plan': the pigs were either treated as a single population, (no sorting), or the lightest 10, 20 and 30 percent of the population were fed a higher digestible P in comparison to the remaining population. The supply of dietary digestible P (g/kg) was determined to meet the average digestible P requirements of the sorted and remaining population at the mid-point BW of each feeding phase.

| Sorting plan | Cumulative P excreted (kg) | | | | | |
|---|---|---|---|---|---|---|
| | Total | | Insoluble | | Soluble | |
| | No sorting | Sorting | No sorting | Sorting | No sorting | Sorting |
| 10% lightest | 17.6 | 26.3 | 5.00 | 7.10 | 12.6 | 19.3 |
| Remaining population | 232 | 220 | 62.5 | 58.8 | 170 | 161 |
| Total | 250 | 246 | 67.5 | 65.9 | 183 | 180 |
| 20% lightest | 38.0 | 54.2 | 10.8 | 14.6 | 27.2 | 39.7 |
| Remaining population | 212 | 191 | 56.7 | 52.3 | 155 | 139 |
| Total | 250 | 245 | 67.5 | 66.9 | 182 | 179 |
| 30% lightest | 59.0 | 82.4 | 16.7 | 22.0 | 42.3 | 60.3 |
| Remaining population | 191 | 160 | 50.8 | 39.8 | 140 | 120 |
| Total | 250 | 242 | 67.5 | 61.8 | 182 | 180 |

A smaller percentage of ‘light’ pigs were supplied less than 85% of their digP requirements at any stage of growth, compared to the equivalent group of the population that were not sorted (**Figure 4.10 – 4.12**).The converse was the case for the ‘remaining’ of the population; a larger percentage of the ‘remaining’ pigs were supplied less than 85% of their digP at any stage of their growth, compared to the equivalent group of the population that were not sorted. Nevertheless, the difference between the sorted and not sorted regimes was higher for the light group compared to the remaining group.

Increasing the size of the ‘light’ group resulted in an increase in their average initial BW and a decrease in the time needed to reach the target BW of 30kg (**Table 4.6**). The average initial BW of the lightest 10, 20 and 30% of the sorted population was 5.5, 4.2 and 3.3 kg lighter than that of the unsorted population and needed 114, 111 and 109 days to reach the average BW of 120 kg. For the remaining 90, 80 and 70% of the population, their average initial BW was 0.9, 1.3 and 1.7 kg heavier and needed 88, 86 and 84 days to reach the average BW of 120 kg, respectively. The CV of the ‘remaining’ group was smaller than for the ‘light’ group. In addition, the smaller the size of each group, the smaller the CV.

The greatest effect of a sorting regime for all the performance variables was when the lightest 30% of the population was sorted (**Table 4.7**). The performance of the sorted ‘light’ group increased compared to the equivalent group of the population when not sorted. The converse was the case for the ‘remaining’ group, as the performance decreased, compared to the equivalent group of the population that were not sorted. The CV of all population performance variables decreased with increasing the size of the ‘light’ group. The CV of the ADG for the sorted pigs increased by sorting, while the CV of the protein and P retained decreased in comparison to the equivalent group of the population that were not sorted.

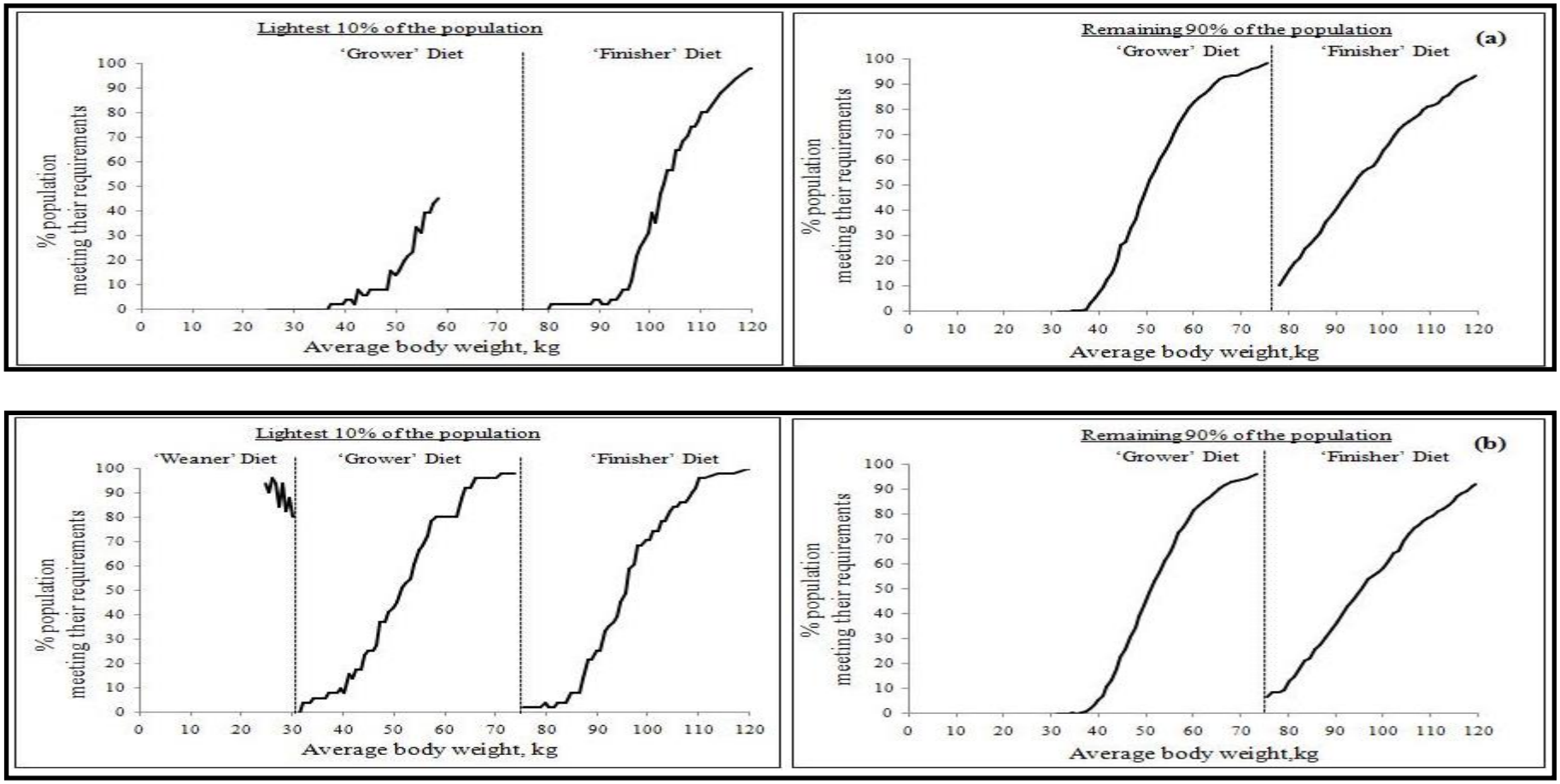


**Figure 4.7** The percentage of the population that met their digestible P requirements when the pigs were either: **(a)** treated as a single population, no sorting/control or; **(b)** the lightest 10 percent of the population were fed a higher digestible P diet and had an extra phase in comparison to the control, because they were fed in accordance to their requirements and vice-versa for the remaining 90% of the population.

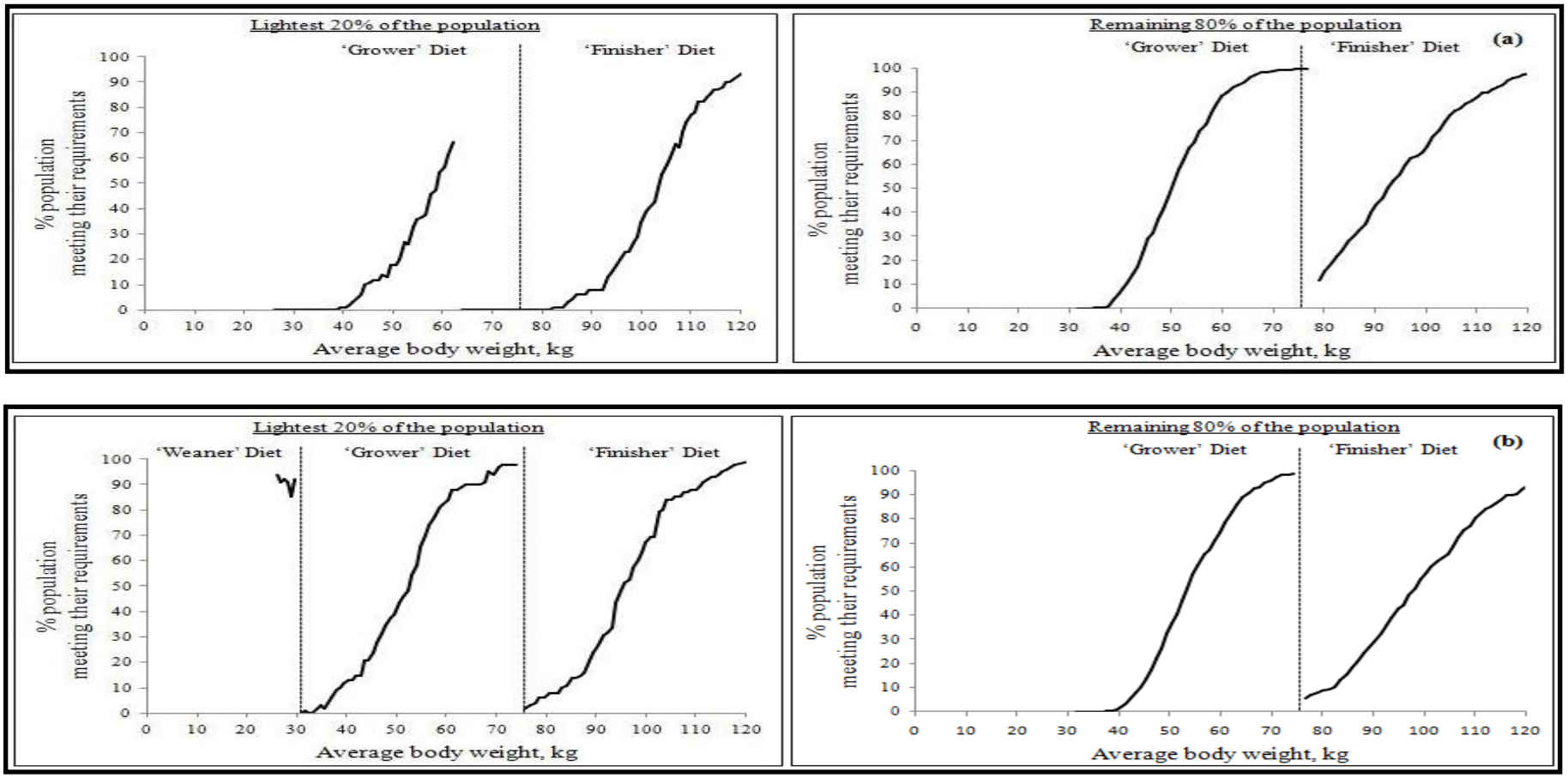


**Figure 4.8** The percentage of the population that met their digestible P requirements when the pigs were either: **(a)** treated as a single population, no sorting/control or; **(b)** the lightest 20 percent of the population were fed a higher digestible P diet and had an extra phase in comparison to the control, because they were fed in accordance to their requirements and vice-versa for the remaining 80% of the population.

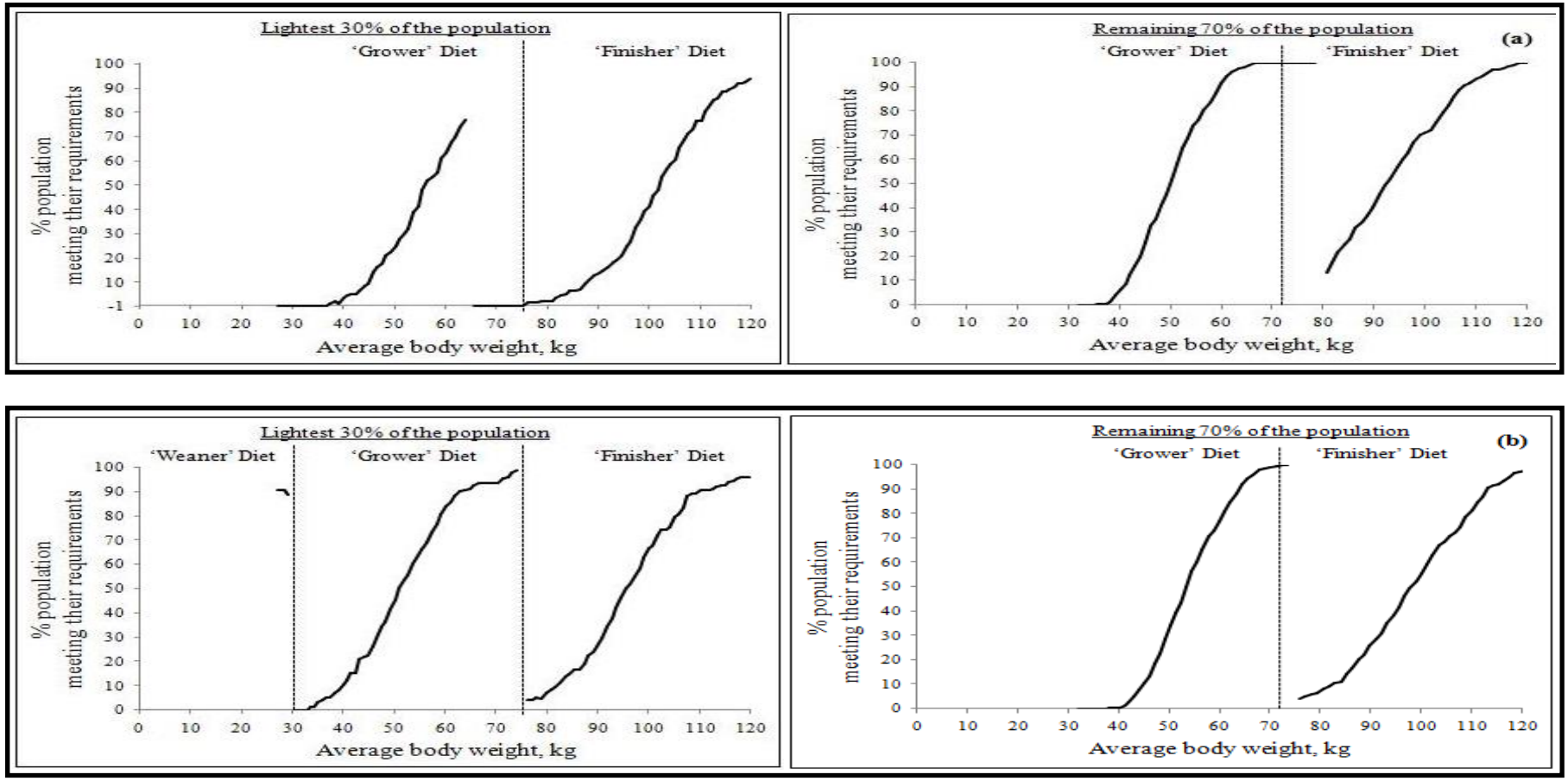


**Figure 4.9** The percentage of the population that met their digestible P requirements when the pigs were either: **(a)** treated as a single population, no sorting/control or; **(b)** the lightest 30 percent of the population were fed a higher digestible P diet and had an extra phase in comparison to the control, because they were fed in accordance to their requirements and vice-versa for the remaining 70% of the population.

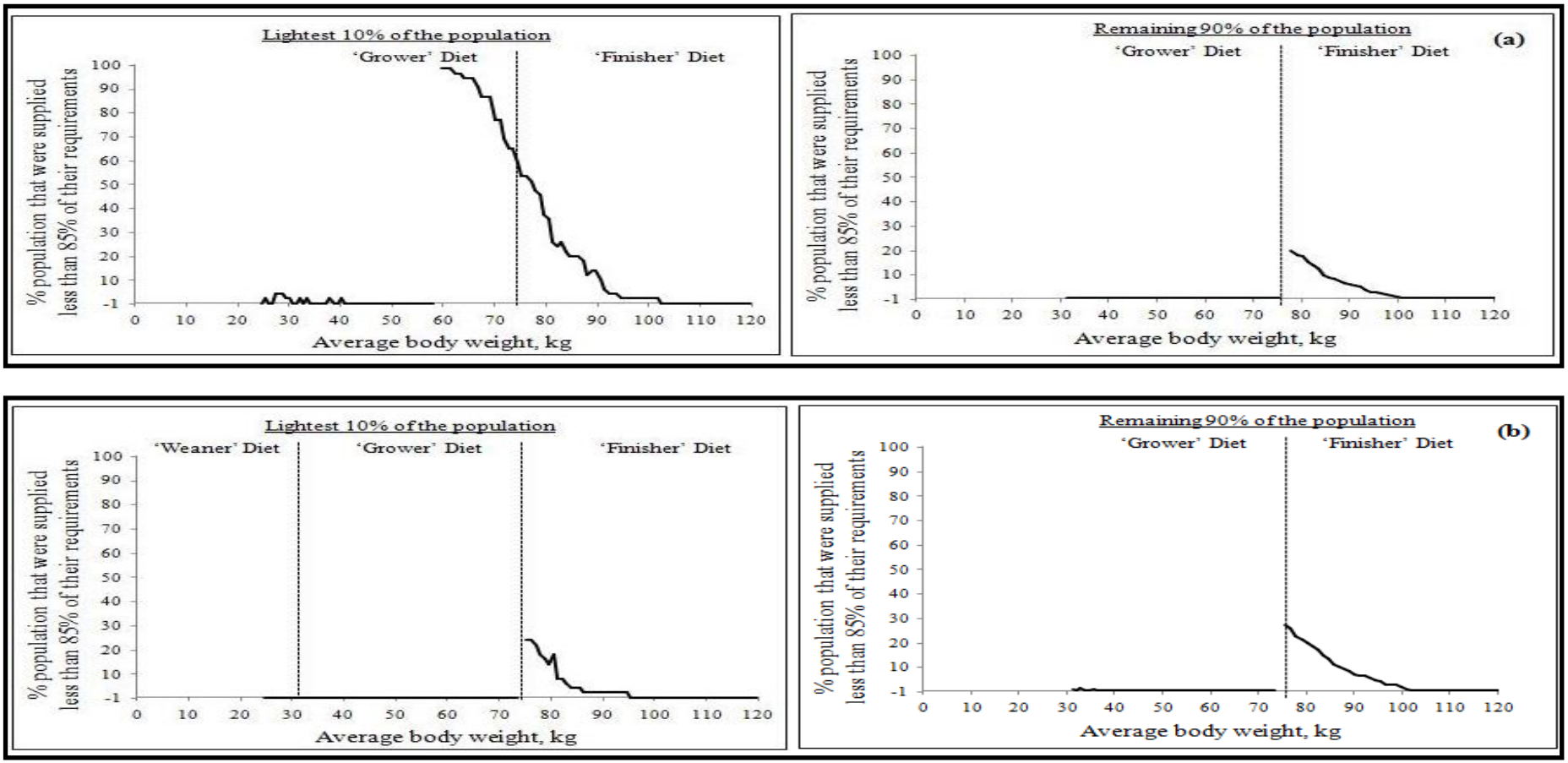


**Figure 4.10** The percentage of the population that were supplied less than 85% of their digestible P requirements when the pigs were either: **(a)** treated as a single population, no sorting/control or; **(b)** the lightest 10 percent of the population were fed a higher digestible P diet and had an extra phase in comparison to the control, because they were fed in accordance to their requirements and vice-versa for the remaining 90% of the population.

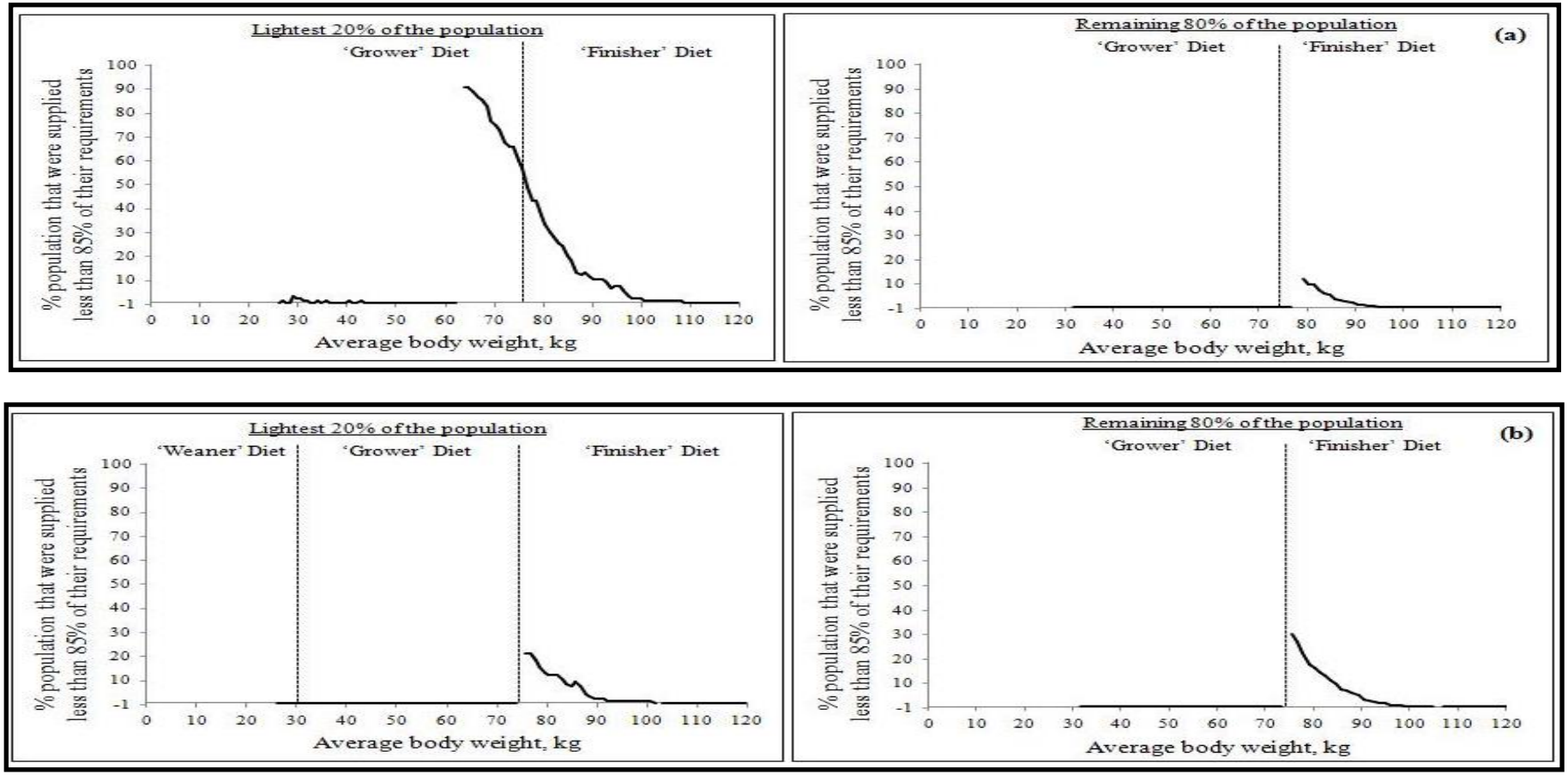


**Figure 4.11** The percentage of the population that were supplied less than 85% of their digestible P requirements when the pigs were either: **(a)** treated as a single population, no sorting/control or; **(b)** the lightest 20 percent of the population were fed a higher digestible P diet and had an extra phase in comparison to the control, because they were fed in accordance to their requirements and vice-versa for the remaining 80% of the population.

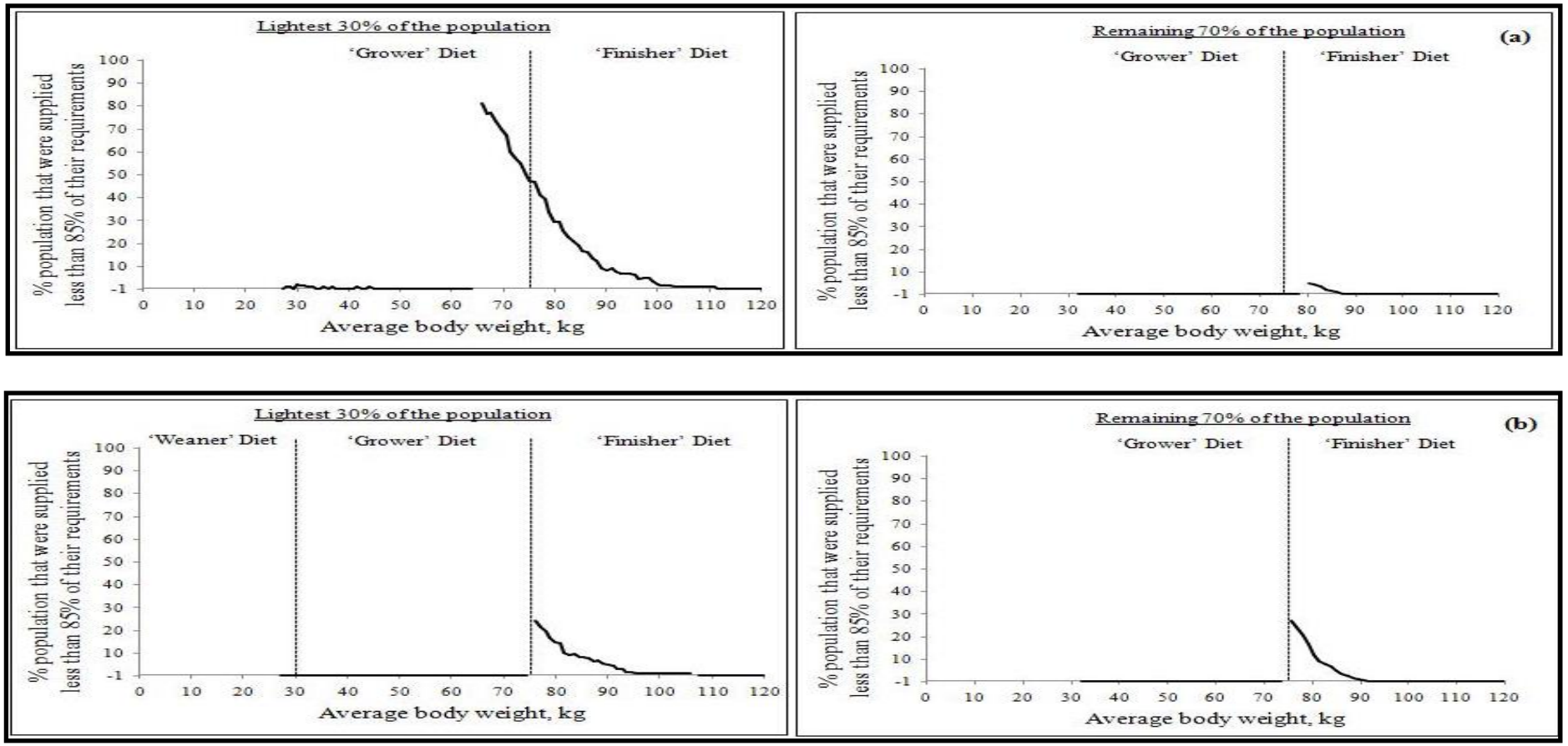


**Figure 4.12** The percentage of the population that were supplied less than 85% of their digestible P requirements when the pigs were either: **(a)** treated as a single population, no sorting/control or; **(b)** the lightest 30 percent of the population were fed a higher digestible P diet and had an extra phase in comparison to the control, because they were fed in accordance to their requirements and vice-versa for the remaining 70% of the population.

**Table 4.6** The initial average BW and the time taken on each of the feeding phases of a 'sorting plan': the pigs were either treated as a single population (no sorting) or the lightest 10, 20 and 30% of the population were fed on a higher digestible P, in comparison to the remaining of the population. The supply of dietary digestible P (g/kg) was determined in order to meet the average digestible P requirements of the sorted and remaining population at the mid-point BW of each feeding phase.

| Sorting Plan | BW range (kg) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | <30 | | | 30 – 74 | | | 75 – 120 | | |
| | Start BW (kg) | | Time taken (d) | Start BW (kg) | | Time taken (d) | Start BW (kg) | | Time taken (d) |
| | Mean | CV | | Mean | CV | | Mean | CV | |
| No sorting | - | - | - | 30.3 | 0.0944 | 47 | 74.9 | 0.1576 | 43 |
| 10% sorting | | | | | | | | | |
| 10% lightest | 24.8 | 0.0626 | 10 | 30.0 | 0.0738 | 55 | 74.5 | 0.1673 | 49 |
| Remaining population | - | - | - | 31.2 | 0.0702 | 45 | 74.5 | 0.1376 | 43 |
| 20% sorting | | | | | | | | | |
| 20% lightest | 26.1 | 0.0681 | 8 | 30.3 | 0.0759 | 52 | 74.9 | 0.1590 | 51 |
| Remaining population | - | - | - | 31.6 | 0.0589 | 44 | 74.5 | 0.1303 | 42 |
| 30% sorting | | | | | | | | | |
| 30% lightest | 27.0 | 0.0707 | 6 | 29.8 | 0.0772 | 51 | 75.1 | 0.1542 | 52 |
| Remaining population | - | - | - | 32.0 | 0.0498 | 43 | 75.0 | 0.1208 | 41 |

**Table 4.7** The effect of a 'sorting' plan on the performance of a population of pigs from 30-120 kg in terms of: 1) ADG (kg/d); 2) protein (Pr) retained (g/day) and 3) P retained. The pigs were either treated as a single population (no sorting) or the lightest 10, 20 and 30 percent of the population were fed a higher digestible P, in comparison to the remaining of the population.

| Sorting plan | ADG (kg/d) | | | | Pr retained (g/d) | | | | P retained (g/d) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No sorting | | Sorting | | No sorting | | Sorting | | No sorting | | Sorting | |
| | Mean | CV | Mean | CV | Mean | CV | Mean | CV | Mean | CV | Mean | CV |
| 10% lightest | 0.811 | 0.1012 | 0.819 | 0.1078 | 149 | 0.13 | 151 | 0.128 | 4.25 | 0.1192 | 4.65 | 0.117 |
| Remaining population | 1.057 | 0.0937 | 1.054 | 0.0944 | 182 | 0.108 | 181 | 0.1074 | 5.72 | 0.1107 | 5.7 | 0.1092 |
| 20% lightest | 0.875 | 0.0997 | 0.879 | 0.1046 | 160 | 0.1258 | 161 | 0.1249 | 4.62 | 0.1186 | 4.95 | 0.1147 |
| Remaining population | 1.072 | 0.0932 | 1.066 | 0.0952 | 183 | 0.1063 | 182 | 0.1057 | 5.81 | 0.1099 | 5.77 | 0.109 |
| 30% lightest | 0.897 | 0.0987 | 0.9 | 0.104 | 163 | 0.1236 | 164 | 0.1168 | 4.77 | 0.1184 | 5.04 | 0.1124 |
| Remaining population | 1.091 | 0.0922 | 1.082 | 0.0993 | 185 | 0.1046 | 184 | 0.1043 | 5.85 | 0.109 | 5.79 | 0.1084 |

## 4.5 Discussion

Developing a stochastic model to predict the consequences of feeding strategies on nutrient excretion of groups of animals is associated with significant challenges. These arise from the fact that the sources of variation in the model inputs need to be described adequately, so that their consequences on model outputs, such as nutrient retention and excretion, can be predicted satisfactorily. This issue cannot be addressed by a deterministic model. Following the framework of Emmans and Kyriazakis (2001), we assumed that variation in model inputs will arise from animal characteristics, feed composition (and hence strategy) and environmental features, such as ambient temperature and group composition. We further assumed that there would be no stochastic variation in the latter component and that the environment will be non-limiting at all stages of the simulation; we appreciate that this is an over-simplification (Wellock et al., 2004).

Stochasticity in animal characteristics was introduced in three genetic parameters (B*, $Pr_m$, and $LPr_m$) in accordance to Ferguson et al (1997), Knap (2000), Pomar et al (2003) and Wellock et al (2004). This allowed for variation in maintenance and growth requirements amongst individuals to be accounted for. In accordance with Kyriazakis (2011) we assumed no measurable genetic variation in nutrient digestibility and net efficiency of nutrient utilization between animals. This approach introduced the challenge of estimating the variation and co-variation in the parameters that were subject to genetic variation (Knap et al, 2003). Currently, there are several methodologies that enable the estimation of these parameters, such as the 'inverse modeling' used by Knap et al. (2003) and Doeschl-Wilson et al. (2005; 2007). The issue is whether the variation or co-variation between these traits can be adequately defined, as frequently estimates derive from relatively small population sizes (Doeschl-Wilson et al., 2005). Brossard et al. (2009) emphasized the importance of accurate knowledge of the individual nutrient requirements, if multiphase sequence plans are used. Without this knowledge, there is a risk of increasing the variability in performance over the intrinsic phenotypic variability in the population.

Although feed composition changed during the course of the simulation according to the investigated feeding strategies, the composition of the feed at any particular point in time was not subject to stochastic variation. This is again a simplification, as feed composition may vary stochastically, due to variation in nutrient composition of the ingredients that compose a feed (Kim et al., 2002) or uncertainty introduced by feed processing or mixing (Groesbeck et al., 2007). Introducing uncertainty in feed composition and environmental features is a long neglected issue in nutrition and metabolism models, and represents our next challenge in model development. The feeds offered to the pigs during the different strategies addressed were assumed to be first limiting in P and that all other nutrients met the requirements of the animals. Even if the latter was not the case for all individuals in the population, pigs would have been able to increase feed intake to meet the requirements for the limiting protein or energy (Kyriazakis and Emmans, 1999). There is no evidence to suggest that the same would happen when P or Ca are the first limiting feed resource (Pomar et al., 2006; Lopes et al., 2009). When the level of P in the diet changed the ratios of total Ca:dig P also changed to those recommended by the UK Industry. These were closer to the ratios recommended by NRC (2012), rather than BSAS (2003) and reflect the uncertainty associated with the appropriate levels of total Ca:digP. There have been recent calls (Selle and Ravindran, 2008; Selle et al., 2011) to suggest that current levels of total Ca in the food are high and maybe associated with poor performance.

### *4.5.1 Phase feeding*

We addressed the consequences of two feeding strategies that aim to minimize P excretion. Both of them were considered to have practical applicability, although simulation models should be able to address treatments beyond the confines of practicality. Phase feeding is the most studied feeding strategy, when aiming to decrease nutrient excretion (Lenis, 1989; Coppoolse et al., 1990; Henry and Dourmad, 1993; Han et al., 1998; Lee et al. 2000; Brossard et al., 2009; Pomar et al., 2011). In theory, the content of the feed in the nutrient whose excretion is aimed to be minimized should change as frequently as possible. There are of course limits on how often this can be achieved without disruption in farm practices, although with the advances of livestock precision farming, the delivery of mixtures between two (basal) feeds to deliver the appropriate amount nutrient in the feed at group or individual level may be possible

(Pomar et al., 2009). Although in principle our model is capable of investigating the consequences of several changes in the feed during a growing period, here we investigated the consequences of two- and three-phase feeding when compared with a single phase feeding. It is unlikely that increasing the number of changes beyond that number would have industry application (van der Peet-Schwering et al., 1996).

Increasing the number of feed changes (feeding phases) resulted in the expected decreases in P excretion, in total, insoluble and soluble P forms. The decreases were more dramatic when the feeding regime changed from one to two phases, rather than from two to three phases. It is likely that the reductions in P excretion follow the law of diminishing returns when the number of feeding phases increases. P excretion was higher using1/3 target, as opposed to 1/2 target, and consequently the reductions in P excretion were higher in the former regime when the feed changes were more frequent. This is consistent with the simulation of Pomar et al. (2011) who found substantial reductions in P excretion through individual precision feeding as opposed to three-phase feeding; the latter met the digP requirements of the average of the population at the start of each phase. These findings cannot be compared directly with literature; when phase feeding has been practiced experimentally both the P and N content of the feed has changed simultaneously (Lenis, 1989; Coppoolse et al., 1990; Henry and Dourmad, 1993), and there is no direct correspondence between the feeds and animals used in the experiments and the simulation. Nevertheless, the former two studies have found a reduction of 6% in P excretion by moving from one to two phases, which is comparable to the reductions achieved here when the same feeding regime applied (7%). The trigger for changes in the feed composition of the different phases used in our simulations was weight, although time could also be used. It is unlikely that the conclusions reached by this study, as far as P excretion is concerned, would be affected by this.

As well as resulting in reduction in P excreted, increases in the number of feed changes resulted in effects on performance: increases in ADG, Pr and P retained, and decreases in FCR. Again these effects were more substantial when the feeding regime changed from one to two phases, rather than from two to three phases. A further consequence of these regimes was the CV of variation in the population for the performances characteristics considered was substantially reduced. This would have significant

economic implications, as there are financial penalties associated with the variability of a batch of pigs at slaughter (Patience et al., 2002; Patience and Beaulieu, 2006). The increases in BW gain were relatively small but associated with very small errors, which suggest that it may be difficult to observe them experimentally. There are no comparable experiments in the literature, but Pomar et al (2009; 2011) simulated the differences in performance between a three phase feeding regime and meeting the digP requirements of the pigs individually through precision feeding. They suggested that there were no differences in performance between these two feeding regimes. This is likely to reflect the fact that a three phase feeding regime already met the requirements of a substantial number of pigs in the population, as suggested here.

The increases in both Pr and P retained through increases in the number of feed changes most likely reflect some of the assumptions made by the model in chapter 2. In the deterministic model it was assumed that the relationship between Pr and P retention was isometric, in accordance with Rymarz et al. (1982), Jongbloed (1987), Hendriks and Moughan (1993), and Manhan and Shields (1998).Therefore, when the pigs are unable to grow P at the maximum rate defined by the genotype, because digP fails to meet their requirements, they will at the same time fail to grow Pr at the rate defined by its genotype, even if the feed amino acid content is non-limiting. This assumption is different from what is currently proposed by NRC (2012). This is perhaps the main reason that our predictions differ from those of Pomar et al (2011) in this respect.

In addition to investigating P excretion, we also investigated two more outputs of interest: the percentage of the population that met the digP requirements and the percentage of the population that were supplied less than 85% of their digP requirements at a particular BW. The first was in accordance with Brossard et al. (2009) and the second in accordance with NRC (2012) who suggested that if pigs are undersupplied with digP by more than 15% of their requirements, this will negatively affect their growth. Both outputs can be related to potentially negative effects of pig performance, as discussed above, but at the same time they may relevant to animal welfare. Jensen et al. (1993) found that even small deviations meeting the requirements of pigs in amino acids can lead to significant increases in exploratory behaviour and activity, and changes in posture. Consequently, Kyriazakis and Tolkamp (2011) have

suggested that such failures in meeting the requirements of the pigs may lead to undesirable behaviors, such as vice (Day et al., 1996). Increasing the number of phase feeding sequences resulted in an increase in the percentage of animals whose digP were met and a decrease in the percentage of population supplied with less than 85% of their requirements at a particular BW. These may have consequences on the welfare of the animals as suggested above, over and above the effects in P excretion.

### *4.5.2 Sorting according to body weight*

The popular use of the all-in/all-out production systems implies that management is important at a group level. Variability within a batch of pigs may result in more time to clear a barn till restocking, or more financial penalties at slaughter. A strategy occasionally used by the pig industry to overcome these adverse effects is to apply sorting of the population of pigs into 'light' and 'remaining' groups and manage these two groups in different finishing pens (Tokach, 2004). Thus, the remaining group could be 'closed out' sooner and restock faster. Sometimes the lighter group can be fed a different feed in order to meet the different nutrient requirements from the remaining pigs. The question is what the consequences of this management strategy are in terms of P excretion and performance.

The simulations suggest that although there are reductions in the cumulative P excreted when the strategy was applied, these were relatively small, when compared to the P excreted by the unsorted situation. The cumulative P excreted reduced by 1.5, 2 and 3%, as the size of the light population increased from 10 to 20 to 30% of the total population, respectively. This resulted from increases in the P excreted by the light population and decreases in the P excreted by the remaining population. For all these simulations we assumed that the feed composition will change only once throughout the growing finishing period, which is equivalent to two-phase feeding. In addition the light pigs were maintained on the nursery feed for a longer period of time before they were switched over to the grower one.

When applying the above strategy the sorted pigs were fed according to the digP requirements of the average of the sorted populations. As a consequence the light pigs received diets of higher digP content and the remaining pigs received diets of lower digP content. The consequence of this was an increase in the performance of the light pigs, in terms of BW gain, Pr and P retained. However, there were smaller decreases in the performance of the remaining sorted pigs compared to the remaining pigs in the unsorted population. These arose from the fact that a smaller number of remaining pigs met their digP requirements throughout the simulation in the sorted scenario. Our findings contrast with those of O'Quinn et al. (2000) and Schinckel et al. (2005; 2007) who suggested that sorting had no effects on the performance of the pigs in the sorted and unsorted populations. However, in these experiments both sorted and unsorted pigs were fed the same diets. Therefore, it is important to appreciate what is aimed to be achieved by any sorting practices. In the experiments of O'Quinn et al. (2000) it is likely that it was hypothesized that any effects on light pigs would arise from the absence of competition, which would put lighter pigs at a disadvantage (Hessing et al., 1994). In our experiment the aim was to reduce the P excreted by the batch of pigs and hence a change in the feeding regime was also deemed necessary. The CV of the ADG for the sorted pigs increased by sorting, probably because the level of under and over-supply of digP was larger in comparison to the unsorted group, where a large percentage of the population were underfed in digP.

As with phase feeding, the application of sorting increased the percentage of the population that met the digP requirements and decreased the percentage of the population that were supplied less than 85% of their digP requirements at a particular BW, but only for the light pigs. This was because the management regime met more closely their requirements as a whole. The converse was the case for the remaining pigs and was a consequence of the content of the feed offered to these pigs being lower when the populations were sorted rather than unsorted.

### 4.6 Future model development and Implications

As discussed above the model assumed that as soon as digP supply to an individual pig was reduced, both P and Pr retention were penalized. It is however, possible that the bones can act as P storage which can be utilized at times of relatively small P deficiency (Henry and Norman, 1984; Hurwitz, 1996; DeLuca, 2008). This has been assumed by the NRC (2012) model.

The model simulations were based on a population of 500 pigs, as this was considered to reflect the size of a pig batch typically grown in the UK. As some of the differences observed in P excretion and performance by the management strategies applied are relatively small, it would be important to know if the effects are due to the population size considered, as discussed previously. However, given the small standard errors associated with the simulated means, this seems unlikely.

The simulations suggest that P excretion was higher when feeding regime targeted the requirements of the first third of the period as opposed to targeting the requirements at the mid-point. As there is a common feeding regime between the phase feeding and the sorting strategies some comparisons between the two can be made; the common feeding regime being a two phase feeding regime when the population of pigs was treated as a whole. Sorting according to BW reduced further the cumulative P excretion. However, like for all management treatments the question is what the economic implications of their application are? Our simulations did not consider this issue and perhaps incorporating an economic module in the model should be one of the areas of its model developments. This has only been done at rough level by other stochastic models.

Besides the economic implications of severely underfeeding digestible P to some individuals in the population, there is also a welfare implication, it is possible that it may lead to behavioural problems (Kyriazakis and Savory, 1997). Jensen et al., (1993) found that very mild deficiencies in dietary nutrients led to an increase in exploratory and other behaviours such as tail biting. If there is a clear quantitative relationship between the degree of nutrient (P) deficiency and behavioural modification, then in theory the current model may be extended to predict the incidence of ‘abnormal’ behaviours in a population of pigs. An alternative programming language besides VBA,

which was used in this thesis, could be the usage of an agent-based programming language, such as ‘NetLogo’ and ‘StarLogo’. These models provide an easier way to simulate a pig with its own growth potential and behaviour, with a capacity to adapt and modify its behaviour in comparison to VBA (Macal and North, 2006). ‘NetLogo’ also has an elegant graphical interface with a 3D visualization that enables mixing agent-based and aggregate representations (Pea and Maldonado, 2005). Not all agent-based programming language are easy to use, from example ‘Repast’ is more powerful and flexible than ‘NetLogo’ but requires extensive use of JAVA programming language (Macal and North, 2006).

The stochastic model developed here overcomes the usual criticisms applied on the limitations of deterministic growth and metabolism livestock models (St-Pierre, 2013). The model is capable of considering the consequences of future management strategies that may develop to reduce P excretion by population of pigs, such as those associated with precision livestock feeding.  This can only be achieved by a stochastic approach.

# Chapter 5. The consequences of introducing stochasticity in nutrient utilization models: the case of P utilization by pigs

## 5.1 Abstract

It is generally accepted that uncertainty in pig system components can influence the mean and variance of the performance of a group of pigs. However, simulation models of nutrient utilization usually ignore this. The objective of this study was to develop a methodology to allow us: (a) to investigate how uncertainty about feed composition (arising from variability in ingredient nutrient content and mixing efficiency) would affect the outputs of a nutrient utilization simulation model, and (b) how such uncertainty would interact with the uncertainty that arises from the genetic traits of individual pigs within a population. We used a Phosphorous (P) intake and utilization model to address these issues. The development of a stochastic model to account for variability in nutrient intake and utilization gave rise to a number of methodological challenges, for example how to generate the variation in both feed composition and pigs, and how to account for correlation between ingredients when modelling the uncertainty associated with mixing efficiency. Introducing variation in the feeding environment and genotype resulted in moderate decreases in the mean digested, retained and excreted P predicted for a population of pigs, and in an increase in their associated CVs. There was also a lower predicted percentage of pigs in the population meeting their requirements during the feeding period under consideration (30-120 kg BW) by comparison with the control scenario (no variation). Due to the assumptions made by the model in the scenarios investigated, uncertainty about feed ingredient composition contributed more to performance variation than uncertainty regarding mixing efficiency. When uncertainty about both feed composition and pig characteristics was considered and pigs were simulated under conditions likely to be encountered in commercial environments, it was uncertainty about feed composition rather than pig genetic characteristics that proved to have the dominant influence on variability in pig performance. Based on these findings, a framework has been developed to take account of uncertainty in relation to the components of pig systems. This framework can be used to investigate the consequences for pig performance of uncertainty as regards several components of the system, namely the pig, its feed and its environment. Such

consequences are likely to have a significant impact on decisions about how to feed pig populations that are subject to uncertainties.

## 5.2 Introduction

Most nutrient utilization simulation models, with a few notable exceptions, are deterministic, i.e. they deal with the performance of the average animal, offered a diet of a certain composition, whilst kept in a relatively constant environment. Some models have dealt with the variation between individual pigs and in aspects of the environment (e.g. Ferguson et al., 1997; Wellock et al., 2004), but none has dealt with uncertainty in feed composition at a particular point in time or over time. There are several reasons why the latter may be important. Feed ingredients may vary substantially in nutrient composition, due to growing conditions, hybrid or variety differences, planting and harvest dates, storage and feed out conditions (Kim et al., 2002). In addition, uncertainty in feed composition may arise from the feed manufacturing process, such as mixing and processing (Traylor et al., 1994; Groesbeck et al., 2007; Pedersen et al., 2007). Whilst several authors have identified such uncertainty in feed composition as a significant contributor to variation in performance (Hendriks et al., 2002; Spiehs et al., 2002; Stein et al., 2009; Weis et al., 2012), it is surprising that none has taken it into account in nutrient utilization models.

In this paper we use a model that predicts the digestion, utilization and excretion of phosphorus (P) by growing and finishing pigs (chapter 2 and 3) to address the challenge of incorporating stochastic variation in system components, namely pig genotype and feed composition, and investigate its consequences on the utilization of this nutrient. The model is capable of incorporating stochastic variation, as we have shown in chapter 4. We use the stochastic model as a case in point on how uncertainty in feed ingredients, inefficiency in mixing and uncertainty in the genetic parameters of individual pigs can affect the outputs of a nutritional model, in terms of digested, retained and excreted P.

## 5.3 Material and methods

The single animal model of chapter 2 that predicts the intake, digestion, utilisation and excretion of P for growing and finishing pigs was used for this purpose. The main inputs to this model are: (1) pig genotype, including initial state, (2) feed composition; and (3) feeding plan. The model outputs for a single pig are: (1) average daily gain and food intake (FI),(2) body composition, including P retained, and (3) soluble and insoluble, and hence total P excreted. An important assumption underlying the model is that the relationship between protein (Pr) and P growth is isometric (Rymarz et al., 1982; Jongbloed, 1987; Hendriks and Moughan, 1993; Mahan and Shields, 1998). Stochastic variation in the model has been included in the animal related inputs and described in detail in chapter 4. Here we introduce stochastic variation in feed ingredient composition, variation in the uniformity of the feed arising from mixing and investigate the interactions of these stochastic introductions with the variation in pig genetic traits.

### *5.3.1 Introduction of stochastic variation in feed ingredient composition and mixing*

For the purpose of this study, only ingredient variation that contributes to variation in phytate (oP), phosphate P (NPP), and calcium (Ca) feed content and plant and microbial phytase activity (PPhy and MPhy) was considered. In principle the model is flexible to incorporate variation in other feed resources, provided that these have been measured. The phosphate in the diet was a combination of plant phosphate (pNPP) and inorganic phosphate (iNPP). The dietary Ca also derived from plant (pCa) and inorganic Ca (iCa) sources. The iCa was sourced from both limestone and inorganic salts i.e. mono and di-calcium phosphate.

Variation in the composition of each feed ingredient into the feed was introduced for P and Ca, by considering the standard deviation (SD) of each ingredient provided by Sauvant et al. (2004), see **Table 5.1**. As far as we are aware, Sauvant et al. (2004) have

**Table 5.1** The mean and standard deviation (SD) of the composition of each feed ingredient included in the grower and finisher feeds, in terms of P and Ca content, and plant phytase activity. Variation in the composition of each ingredient was taken from Sauvant et al. (2004), except in the case of plant phytase activity, which was taken from Viveros et al. (2000) and Steiner et al. (2007).

| Ingredient | Total P, g/kg | | Ca, g/kg | | Plant phytase, FTU/kg | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD |
| Barley | 3.40 | 0.300 | 0.700 | 0.400 | 540 | 153 |
| DDGS | 6.40 | 1.40 | 3.30 | 1.20 | N/A | 0 |
| Potato Protein conc. | 4.00 | 1.20 | 2.90 | 2.80 | N/A | 0 |
| Limestone granules | 0 | 0 | 36.5 | 1.50 | 0 | 0 |
| Mono-calcium phosphate | 19.5 | 1.50 | 23.0 | 1.00 | 0 | 0 |
| Rapeseed meal | 11.4 | 0.900 | 8.30 | 1.30 | 10.0 | 20.0 |
| Soybean meal | 6.20 | 0.500 | 3.40 | 0.900 | 20.0 | 40.0 |
| Wheat | 3.20 | 0.300 | 0.700 | 0.300 | 460 | 100 |
| Wheat feed | 3.60 | 0.100 | 0.900 | 0.400 | 3080 | 400 |

published the largest publicly available data base of composition of feed ingredients. However, for some feed ingredients the number of samples used to calculate their mean and SD values is small, and these values should be used with caution. Even though the Sauvant et al. (2004) feed tables provided the PPhy activity (FTU) for all ingredients, they did not provide the SD associated with this. Therefore, variations of ingredient plant phytase activity were derived from Viveros et al. (2000) and Steiner et al. (2007) as they provided the PPhy activity of each ingredient and it's SD. In addition, variation in MPhy supplementation was derived from Akinmusire and Adeola (2009); a SD of 300 FTU per 1000 FTU was assumed to reflect the variation in supplemented MPhy activity.

A stochastic Monte-Carlo simulation was used, to investigate the effect of ingredient variation. The inputs of the Monte-Carlo simulation were: (1) mean and; (2) SD, in each investigated chemical component, for each dietary ingredient (oP, pNPP, pCa and PPhy) or supplement (iNPP, iCa, MPhy). The concentration of most chemical components in plant-based feedstuffs fits an approximately normal distribution (Weiss, 2004), therefore, the use of SD is the most appropriate measure of dispersion.

Using the Monte-Carlo methodology, 500 feeds for each scenario considered were drawn at random, from the above distribution. Once the chemical content of each ingredient (g/kg ingredient or FTU/kg ingredient), for each feed was established, this was multiplied with the ratio of the ingredient's contribution in the feed. The addition of each chemical content of each ingredient resulted in the $oP_i$, $pNPP_i$, $iNPP_i$, $pCa_i$, $iCa_i$ g/kg and $PPhy_i$ and $MPhy_i$ content for each feed.

The goal of feed mixing is to evenly distribute all ingredients and nutrients throughout the entire batch of feed (Groesbeck et al., 2007). A uniform mixture will supply the animal with a balanced diet, ensuring proper nutrient consumption and maximizing performance. A coefficient of variation (CV) of 10% or less for salt or another minor feed ingredient has been adopted as the industry standard to represent a uniformly mixed feed (Traylor et al., 1994; Herrman and Behnke, 1994). Salt is the most common ingredient used to evaluate mixer efficiency (Groesbeck et al., 2007) and it usually

represents 1% of 'conventional' feeds. Therefore, in theory ingredients that makes up a significant percentage of the feed (i.e. wheat), will have a much lower CV due to mixing and this needs to be taken into account when formulating rations.

The modelling approach is based on Bayes Theorem, describing how the conditional probability of a cause (mixing effect of an ingredient in a diet) for a given observed outcome (CV of an ingredient in a diet assuming perfect mixing) can be computed from knowledge of the probability of a cause and the conditional probability of the outcome. Bayes formula for conditional probability can be expressed as:

$$P(diet|mixing) = \frac{P(mixing)*P(mixing|diet)}{[P(mixing)*P(mixing|diet)]+ [P(no\ mixing)*P(no\ mixing|diet)]} \quad \textbf{(5.1)}$$

where, $P(diet|mixing)$ is the final estimate of each ingredient in a diet due to mixing, $P(mixing)$ is the initial or prior estimate of probability assuming perfect mixing, $P(mixing|diet)$ is the probability of a mixing effect of each ingredient given their proportion in the initial mixing (conditional probability), $P(no\ mixing)$ is the initial or prior estimate of probability assuming no mixing and $P(no\ mixing|diet)$ is the probability of a non-mixing effect of each ingredient given their proportion in the initial mixing (conditional probability). An assumption of Bayes' Theorem is that the predictor variables be independent (Tucker et al., 1997). In the context of the present chapter the ingredients that compose the diet are independent; therefore the Bayes' Theorem can be used successfully.

In order to quantify the later statement, it was first necessary to set the target ingredient composition of the selected feed, assuming perfect mixing. Then based on this feed, a distribution function was specified, where the probability of occurrence of each ingredient equalled their proportion in the target feed. A repeated random sampling from the distribution that specifies the target feed was carried out, and a random feed was constructed from these samples. When the number of samples increased, the actual composition of the random feed automatically moved closer to that of the feed with perfect mixing, i.e. low number of samples demonstrated an inefficient mixing process and a high number an efficient mixing process.

The number of samples needed to achieve the required level of mixing was achieved through a Monte Carlo approach. Monte Carlo simulations with the pig model were carried out where for each run a separate random feed was constructed. After the simulations, the mean and CV of the proportion of each feed ingredient were specified. The CV of some minor ingredient, e.g. limestone, were used as an indicator of the efficiency of mixing. We run the pig model 500 times and for each run we used a random feed based on 3000 samples. We found that the CV of limestone content in these feeds was approximately 20%. We considered this as inefficient mixing. To simulate a better mixing process we run the pig model 500 times with 6000 feed samples for each run. We got approximately 10% CV in limestone content, which is considered an efficient mixing in accordance to industry standards (Traylor et al. 1994; Herrman and Behnke, 1994). Unlike the variation of ingredient composition, in which only the P, Ca, MPhy and PPhy activity was affected, mixing introduced variation in energy and amino acid (lysine) contents in the feeds, due to the variation in ingredient content.

### *5.3.2 Introduction of stochastic variation in pig genotype and start weight*

The genetic parameters considered to vary between pigs were protein at maturity ($Pr_m$), lipid to protein ratio at maturity ($LPr_m$) and the scaled maturity rate (B*), in accordance with Ferguson et al. (1997), Knap (2000), Pomar et al. (2003), Wellock et al. (2004).

The mean and SD of $Pr_m$ was estimated from the study of Knap et al. (2003) to be 35 and 4.38 kg, respectively. The mean and SD of B* was estimated at 0.0392 and 0.0078 kg/day, respectively, from Brossard et al. (2009), who in turn derived it from the data of Rivest (2004). Finally the mean and SD of $LPr_m$ were derived from Knap and Rauw (2008) to be 1.50 kg/kg and 0.315 kg/kg, which were in turn adapted from Doeschl-Wilson et al. (2005). The initial BW of the pigs (BW0) was in accordance with the methodology of Wellock et al. (2004), having an average BW0 of 30 kg and their chemical composition was calculated assuming that the pigs had their ideal composition set by the genotype (Emmans and Kyriazakis, 2001). The values of B*, $Pr_m$, and $LPr_m$ were assumed to be uncorrelated and normally distributed (Ferguson et al., 1997; Knap, 2000; Pomar et al., 2003; Wellock et al., 2004).

### *5.3.3 Simulation scenarios considered*

The model was run between 30 to 120 kg average pig BW. The genetic parameters that represent a current genotype were used to derive the requirements for net energy (MJ), SID lysine (g) and digestible P (digP) (g) per day. The requirements were derived in accordance with chapter 2. The composition of the feed offered to the pigs changed only once during the simulations at 75 kg BW, in order to meet the nutrient and energy requirements of the average pig. In all scenarios used, the average digP requirements were at the mid-point BW of each feeding period, which was at 52 kg for the grower period (30-75 kg BW) and 98 kg BW for the finisher period (76-120 kg). The net energy and lysine feed contents were chosen so that the feed was first limiting in digP during each period under consideration, while other nutrients (energy and lysine) slightly exceeded the average requirements, see **Table 5.2**.

**Table 5.2** Ingredient and calculated chemical composition of the 'conventional' and 'co-product' based feeds offered to growing (30-75 kg BW) and finishing (76-120 kg BW) pigs.

| | 'Conventional' feed | | 'Co-product' feed | |
|---|---|---|---|---|
| Ingredient, % | Growing | Finishing | Growing | Finishing |
| Barley | 16.1 | 37.4 | - | 7.80 |
| Wheat | 50.0 | 40.8 | 37.0 | 33.9 |
| Wheatfeed | - | - | 5.00 | 5.00 |
| DDGS Wheat | - | - | 25.0 | 25.0 |
| Soybean 47% | 21.3 | 6.35 | 12.4 | 13.4 |
| Rapeseed ext. | 8.00 | 12.5 | - | - |
| Potato Protein | - | - | 13.5 | 7.30 |
| Soya oil | 2.16 | 0.500 | 4.06 | 2.00 |
| Limestone | 0.800 | 0.790 | 0.900 | 0.800 |
| Mono-calcium phosphate | 0.300 | 0.110 | 0.100 | - |
| Sodium Chloride | 0.740 | 0.740 | 0.660 | 0.590 |
| Premix[1] | 0.650 | 1.40 | 0.940 | 1.52 |
| | | | | |
| Calculated composition[2] | | | | |
| Net energy, MJ/kg | 10.0 | 9.60 | 10.0 | 9.60 |
| Protein, g/kg | 202 | 157 | 223 | 181 |
| Total lysine, g/kg | 12.8 | 9.60 | 12.8 | 9.60 |
| Total Ca, g/kg | 6.40 | 6.60 | 6.30 | 6.50 |
| Total P, g/kg | 5.50 | 5.70 | 5.50 | 5.50 |
| Digestible P, g/kg | 3.20 | 2.70 | 3.20 | 2.70 |

[1] Provided sufficient quantities of vitamins and micro-minerals.

[2] Calculated compositions from Sauvant et al. (2004) feed tables.

#### *5.3.3.1 Simulations for variation in feed ingredient content and mixing efficiency*

We first considered stochastic variation in ingredient composition and subsequently stochastic variation resulting from feed mixing. The effects of these variations were considered on either a 'conventional' feed or a feed based on 'co-products' (**Table 5.2**). The 'co-product' based feed was chosen in order to consider the consequences of higher inherent variation in ingredient composition (Sauvant et al., 2004). These feeds were used as a case point to investigate the effect of ingredient variation and/or mixing on P retention and excretion.

The experimental design addressed by the simulations was a 2 x 2 x 3 factorial design of two feed compositions ('conventional' or 'co-product' based feeds), variation in ingredient composition (with or without) and variation in mixing (no mixing effect, efficient or inefficient mixing). At this stage the genetic parameters of the population remained constant and only the variation in feed ingredients and different mixing effects have been examined. Therefore, 500 Monte Carlo iterations were used to generate each scenario described above.

For the 'conventional' feed scenario the grower and finisher feeds were formulated on a least cost formulation (LCF) basis. For each stage requirements were specified for 13 nutritional parameters, with the most important being: net energy (MJ/kg); crude protein; SID lysine; and minerals including total Ca, total P and digP (g/kg). Seventeen typical ingredients used in UK feed mills were considered; the Sauvant et al. (2004) feed tables were used to determine nutritional composition and digestibility values of these ingredients. Information on ingredient prices for most ingredients was obtained from the Public Ledger (Agra-net, 2013) with specific information on prices for minerals and amino acids provided by Premier Nutrition (Rugeley, Staffordshire, UK).

An Excel solver based linear optimisation tool, was used to formulate separate feeds when optimising for LCF. Using the Solver function, the inclusion of all ingredients added to 100% and the derivedfeed reached or exceeded the target nutrient values

specified at the lowest possible price, without exceeding the specified inclusion limits. The ‘co-product’ based feed did not follow a least cost methodology, because it was forced to contain ingredients with large inherent variation in P and Ca, irrespective of the cost. Therefore distillers dry grain solubles (DDGS) and potato protein concentrate were used. This ‘co-product’ feed formulation had a similar chemical composition with the ‘conventional’ one, see **Table 5.2**.

#### *5.3.3.2 Simulations for variation in both feed composition and pig genetic parameters*

The experimental design addressed was a 2 x 2 x 3 factorial design of two feed compositions (‘conventional’ or ‘co-product’ based feeds), variation in feed due to a mixing process efficiency and ingredient composition (with or without variation in feed) and different degrees of variation in pig genetic variables (no variation, ‘low’ and ‘normal’ variation). The main difference of this experimental design in comparison to the previous one was that genetic parameter variation and variation in feed were included for both the ‘conventional’ and ‘co-product’ based feed.

In accordance with the methodology of Pomar et al. (2003), we compared populations with different between-animal genetic variation. Three populations were generated having 0, 0.5 and 1 times the estimated genetic variation of the above reference population. Reducing variation in the genetic parameters to 0.5 of the current estimates, is consistent with industry desire to increase genetic uniformity amongst commercial pigs (Sullivan, 2007). A 500 Monte Carlo iteration was applied for each scenario, having a unique combination of the parameters BW0, $Pr_m$, $LPr_m$ and B*. The populations addressed in the 0.5 and 1 scenarios differed only in SD of the distribution of the means of the genetic variables. Each of those 500 diets of the ‘conventional’ or ‘co-product’ based feed scenario was randomly fed to one of the 500 pigs.

## 5.4 Results

### *5.4.1 Simulation outputs*

From the generated simulated populations, which were fed according to the above scenarios, the following outputs were calculated: the population mean and SD for total P digested, excreted and retained per day; and the percentage of the population that had their digP requirements met throughout the BW period 30-120 kg of the population. Detailed descriptions of the above calculations are found in chapter 4.

### *5.4.2 Variation in feed ingredient composition and in mixing efficiency*

As expected the introduction of variation due to mixing increased the CV of the mean content of an ingredient in the resulting feeds (Table 5.3). The higher the percentage contribution of an ingredient in the feed, the lower its CV associated with the mean content of the ingredient in the resulting simulations, when mixing efficiency was introduced, see **Table 5.3**. The 'co product' based feeds had a higher CV associated with the mean content of each ingredient, than the 'conventional' feeds. In some cases introduction of inefficient mixing in a co-product based feed increased the CV associated with the mean content of a minor ingredient dramatically; for example up to ~60% for mono-calcium phosphate. This introduces enormous uncertainty in the resulting feed compositions, and as will be seen below it has important consequences on system outputs.

Introducing variation in ingredient composition resulted in a moderate decrease in the mean digP input, P retained and P excreted by the population of pigs offered 'co-product' based feeds, see **Table 5.4**. On the contrary, there were no substantial changes (less than 0.5% reductions) in these outputs when the population of pigs was offered 'conventional' based feeds that included variation in ingredient composition.

**Table 5.3** The effect of feed mixing (efficient and inefficient mixing) on the mean content and coefficient of variation (CV) of each ingredient in the resulting feed. The feeds were either 'conventional' or 'co-product'- based and offered to growing (30-75 kg BW) and finishing (76-120 kg BW) pigs; the means and CV are based on 500 Monte Carlo simulations.

| Ingredient | Efficient Mixing | | | | | | | | Inefficient mixing | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 'Conventional' feed | | | | 'Co-product' feed | | | | 'Conventional' feed | | | | 'Co-product' diet feed | | | |
| | Growing | | Finishing | | Growing | | Finishing | | Growing | | Finishing | | Growing | | Finishing | |
| Ingredient, % | Mean | CV | Mean | CV | Mean | CV | Mean | CV | Mean | CV | Mean | CV | Mean | CV | Mean | CV |
| Barley | 16.1 | 2.19 | 37.4 | 1.20 | - | | 7.78 | 3.33 | 16.1 | 4.25 | 37.4 | 2.48 | - | - | 7.82 | 6.42 |
| Wheat | 50.0 | 0.970 | 40.8 | 1.22 | 37.0 | 1.29 | 34.0 | 1.31 | 49.9 | 1.46 | 40.8 | 2.34 | 37.0 | 2.45 | 33.9 | 2.50 |
| Wheatfeed | - | - | - | - | 5.03 | 4.48 | 4.97 | 4.40 | - | - | - | - | 4.98 | 8.21 | 4.99 | 7.89 |
| DDGS Wheat | - | - | - | - | 25.1 | 1.76 | 25.0 | 1.75 | - | - | - | - | 25.0 | 3.08 | 25.0 | 3.11 |
| Soybean 47% | 21.3 | 1.83 | 6.33 | 3.67 | 12.4 | 2.63 | 13.4 | 2.50 | 21.3 | 3.71 | 6.35 | 7.37 | 12.4 | 4.78 | 13.4 | 4.67 |
| Rapeseed ext. | 8.02 | 3.58 | 12.5 | 4.27 | - | - | - | - | 7.98 | 6.35 | 12.5 | 4.97 | - | - | - | - |
| Potato Protein | - | - | - | - | 13.5 | 2.44 | 7.31 | 3.52 | - | - | - | - | 13.5 | 4.51 | 7.29 | 6.44 |
| Limestone | 0.800 | 11.2 | 0.790 | 11.1 | 0.900 | 10.5 | 0.800 | 10.5 | 0.800 | 19.0 | 0.790 | 19.4 | 0.900 | 19.1 | 0.810 | 19.6 |
| MCP | 0.300 | 18.5 | 0.110 | 28.8 | 0.100 | 31.9 | - | - | 0.300 | 33.5 | 0.110 | 55.4 | 0.100 | 58.8 | - | - |
| Other[1] | 3.54 | 5.10 | 2.09 | 6.71 | 6.09 | 4.00 | 6.79 | 3.87 | 3.56 | 9.22 | 2.12 | 12.5 | 6.12 | 7.43 | 6.81 | 6.81 |

[1] Premix, Soya oil and sodium chloride.

**Table 5.4** The effect of variation in ingredient composition (with (yes) or without (no)) and mixing (no mixing (NM) efficient (E) or inefficient (I) mixing) of a 'conventional' and a 'co-product' -based feed on the mean and coefficient of variation (CV) of P digested, excreted and retained. The results are the outcomes of 500 simulations.

| Treatment | | P absorbed into the blood-stream, g/day | | | | P retained, g/day | | | | P excreted, g/day | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mixing | Variation in ingredient composition | 'Conventional' feed | | 'Co-product' feed | | 'Conventional' feed | | 'Co-product' feed | | 'Conventional' feed | | 'Co-product' feed | |
| | | Mean | CV, % | Mean | CV, % | Mean | CV,% | Mean | CV, % | Mean | CV, % | Mean | CV, % |
| NM | No | 7.07 | 0 | 7.07 | 0 | 5.82 | 0 | 5.83 | 0 | 7.44 | 0 | 7.45 | 0 |
| NM | Yes | 7.06 | 4.20 | 6.80 | 9.71 | 5.83 | 2.44 | 5.59 | 7.27 | 7.46 | 5.61 | 7.20 | 9.04 |
| E | No | 6.98 | 3.13 | 6.87 | 2.08 | 5.78 | 2.05 | 5.73 | 1.58 | 7.35 | 2.80 | 7.19 | 2.05 |
| E | Yes | 6.97 | 5.36 | 6.86 | 10.1 | 5.75 | 3.61 | 5.62 | 7.36 | 7.37 | 6.28 | 7.29 | 10.3 |
| I | No | 6.90 | 6.23 | 6.79 | 4.52 | 5.71 | 4.63 | 5.67 | 3.88 | 7.26 | 6.08 | 7.26 | 6.37 |
| I | Yes | 6.89 | 7.73 | 6.80 | 11.1 | 5.68 | 5.55 | 5.57 | 8.27 | 7.28 | 8.04 | 7.37 | 11.0 |

Introducing variation in ingredient composition resulted in the expected increase in the CVs of the model outputs by the population of pigs offered either the 'conventional' or 'co-product' based feeds, with the 'co-product' based feeds leading to approximately twice as high CVs than the 'conventional' based feeds. The reason for the lower P digested, retained and excreted by pigs on the co-product based feeds in comparison to the 'conventional' feeds was due to the higher variation in the supply of P, Ca and phytase activity to the pigs. The model does not allow pigs to compensate for the reduced P supply by increasing their FI on low P feeds, as there is no evidence in the literature that pigs are able to do so (Pomar et al., 2006; Lopes et al., 2009). The feeding regime was such that the feed was formulated to undersupply pigs with P during the early stages and oversupply them during the latter stages of the feeding phase (Chapter 4). Due to the variation introduced by ingredient variation, a larger number of pigs met their digP requirements at the earlier stages of feeding the 'co-product' based feeds, because more P was supplied than planned. The converse was the case during the latter stages of the feeding phase, where a number of pigs were undersupplied with P. Because more P (in g/day) is required as pigs grow (BSAS, 2003), i.e. at the latter stages of each feeding phase, this meant that overall less P was supplied and retained on the 'co-product' based feeds than the 'conventional' feeds.

Variation in mixing efficiency also slightly reduced the average digP intake, P retained and P excreted by the population of pigs, offered either the 'conventional' or 'co-product' based feeds and increased their associated CVs. The decrease in the model outputs and the increase in their associated CVs was twice as much when mixing was less efficient, than when it was efficient. The reasons for the reduced average dig P intake, retained and excreted when mixing variation in the feeds was introduced are identical to those detailed above, when the consequences due to the introduction of variation in ingredient content was accounted for. The effects of introducing variation in feed ingredient and mixing efficiency were not additive, following the principles of error propagation. For example, there was a 4.1% decrease in the P retained when the population was given a 'co-product' based feed that included variation in ingredient composition, and 2.7% reduction in the same output when this arose from simulations that included inefficient mixing. The reduction in the P retained was 4.5 % when both variation in ingredient composition and inefficient mixing were included in the same feed.

The percentage of the population meeting their digP requirements increased with increasing BW during both feeding phases. Feeding a 'co-product' based feed resulted in a lower percentage of the population meeting their digP requirements throughout the feed phase. This was because some of the feeds offered to the pigs never contained adequate P. The increase in the percentage of the population meeting their requirements was more gradual in the 'conventional' (**Figure 5.1**) as opposed to the 'co-product' based feeds (**Figure 5.2**). In all cases an inefficient mixing process slightly increased the number of pigs that met their requirements during the first half of each feeding phase. During the second half of each feeding phase it was the efficient mixing process that greatly increased the number of pigs that met their requirements. An appreciable percentage of pigs (~15%) were still not meeting their digP requirements by the end of each of the feeding phase, when they were offered feeds that resulted from an inefficient mixing process, see **Figure 5.1 - 5.2**.

A similar picture was seen when the feeds included variation due to the ingredient composition, with the main difference being the considerably lower percentage of the population meeting their digP requirements when fed a 'co-product' (**Figure 5.4**) in comparison to a 'conventional' based feed (**Figure 5.3**). The addition of variation due to the mixing process to the ingredient variation increased further the percentage of pigs that met their requirements during the first half of each phase, in comparison to when there was only variation due to the mixing process, and vice-versa during the second half of each phase. This was the case when the feeds offered to the pigs were based on conventional ingredients only; the lowest percentage of pigs meeting their digP requirements was seen when the feeds were based on co-products pigs offered feeds that included variation due to both ingredients and mixing process. An appreciable percentage of pigs (~15%) were still not meeting their digP requirements by the end of each of the feeding phase when the feeds included variation due to both ingredients and inefficient mixing, for 'conventional' based diets. Only 87% and 60% of the pigs managed to achieve their digP requirements during the finisher period in these cases for 'conventional' (**Figure 5.3**) and 'co-product' -based feeds (**Figure 5.4**) respectively, when the mixing process was inefficient.

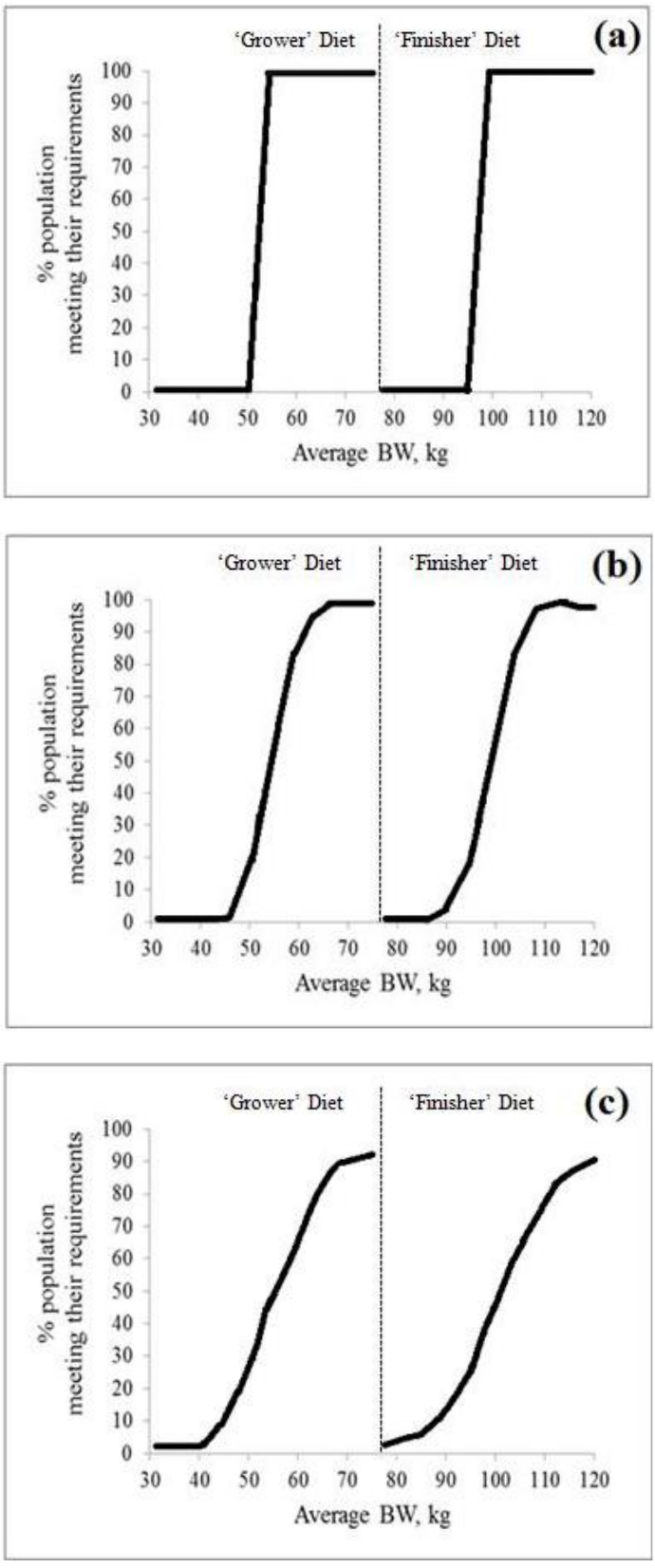


**Figure 5.1** The percentage of the population of pigs that met their digestible P requirements over the average BW range 30 to 120 kg, whilst fed 'conventional' based feed, either **(a)** with no variation or; **(b)** with variation due to an efficient or; **(c)** an inefficient mixing process. All pigs were assumed to be identical in the genetic parameters that defined their growth characteristics.

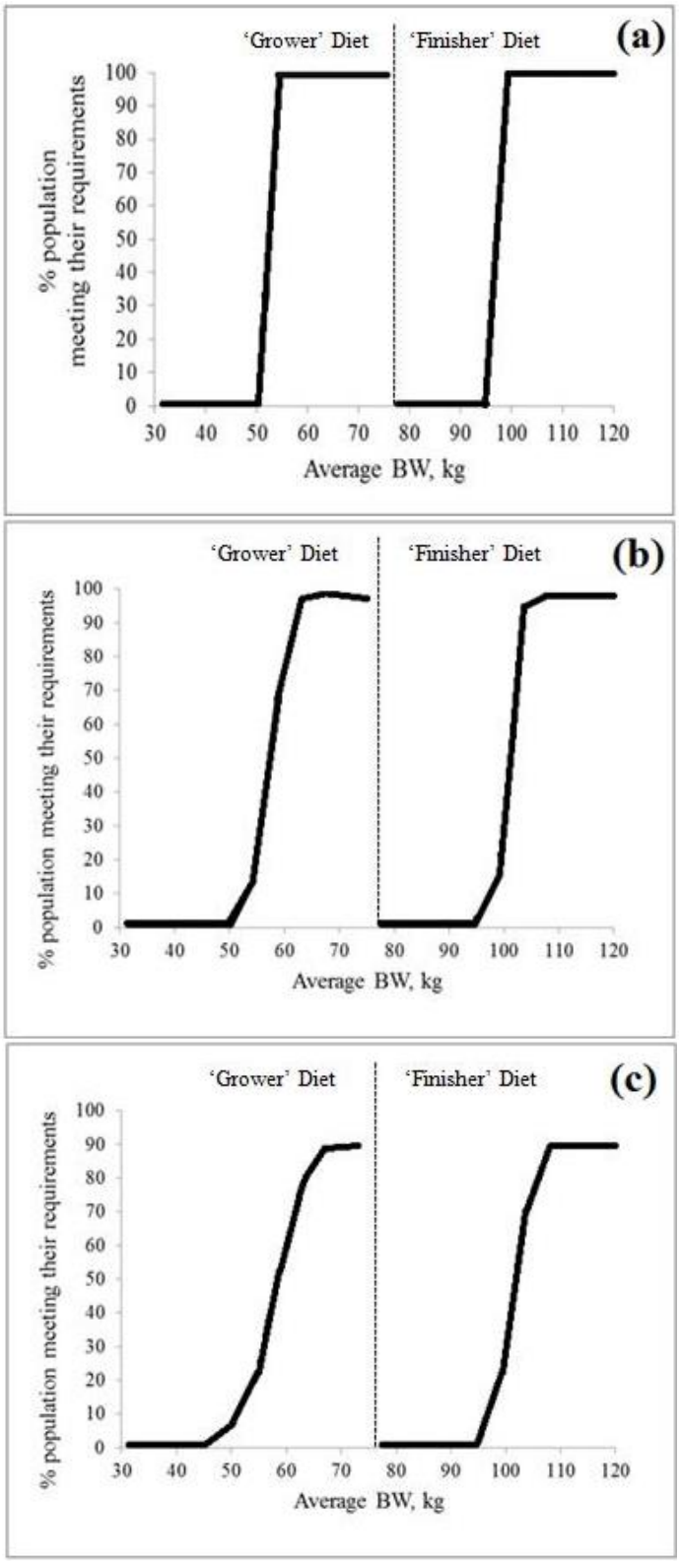


**Figure 5.2** The percentage of the population of pigs that met their digestible P requirements over the average BW range 30 to 120 kg, whilst fed 'co-product' based feed, either **(a)** with no variation or; **(b)** with variation due to an efficient or; **(c)** an inefficient mixing process. All pigs were assumed to be identical in the genetic parameters that defined their growth characteristics.

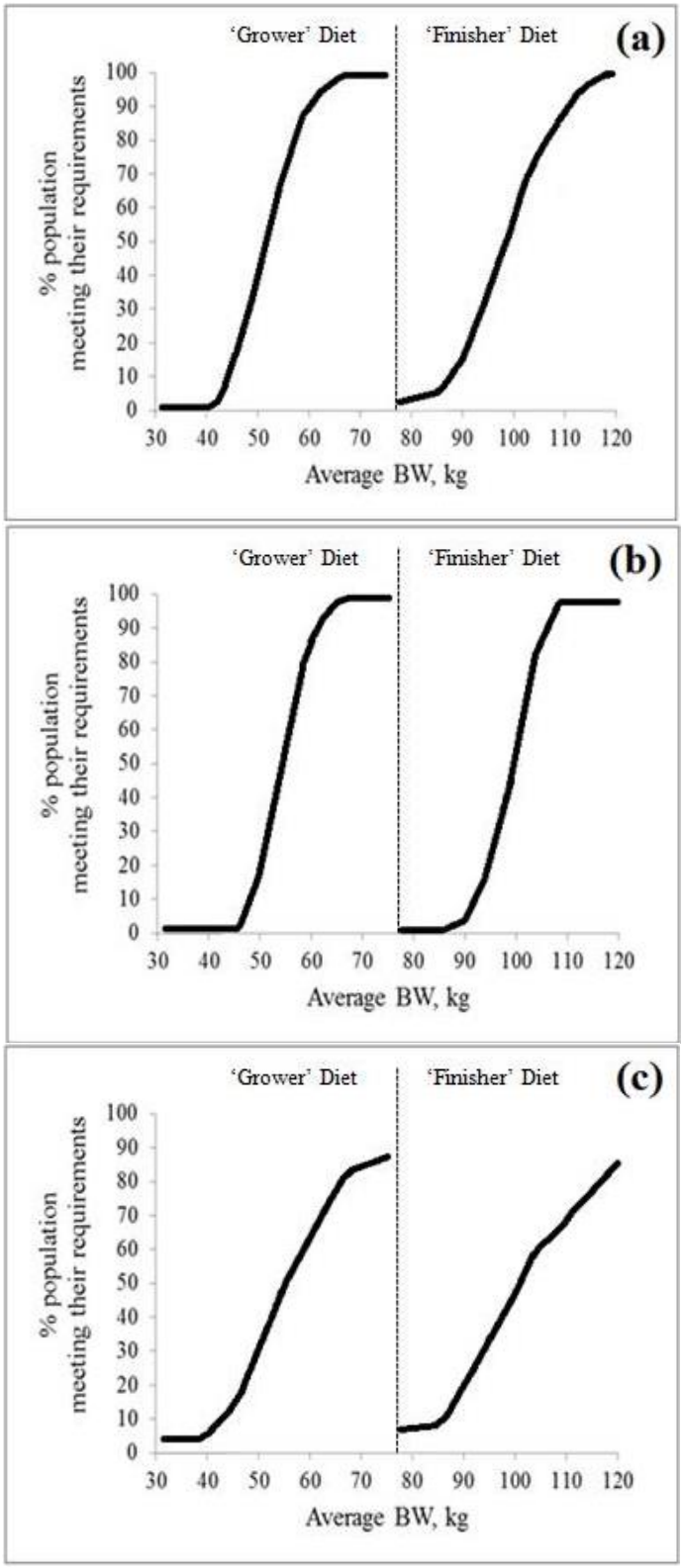


**Figure 5.3** The percentage of the population of pigs that met their digestible P requirements over the average BW range 30 to 120 kg, whilst fed 'convectional' based feed, either **(a)** with variation in ingredient composition or; with variation in ingredient composition and variation due to the mixing process – **(b)** efficient or **(c)** inefficient mixing process. All pigs were assumed to be identical in the genetic parameters that defined their growth characteristics.

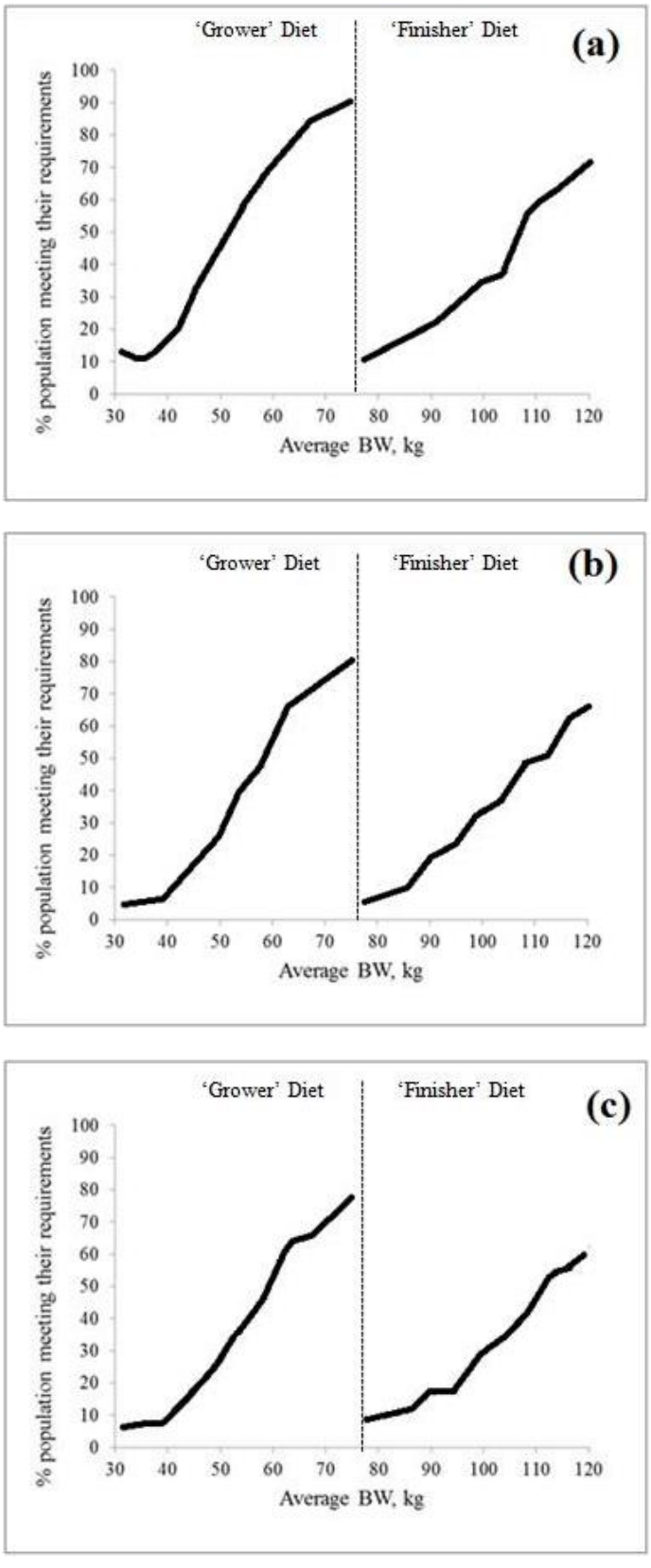


**Figure 5.4** The percentage of the population of pigs that met their digestible P requirements over the average BW range 30 to 120 kg, whilst fed 'co-product' based feed, either **(a)** with variation in ingredient composition or; with variation in ingredient composition and variation due to the mixing process – **(b)** efficient or **(c)** inefficient mixing process. All pigs were assumed to be identical in the genetic parameters that defined their growth characteristics.

### *5.4.3 Variation in feed ingredient composition, mixing efficiency and pig genetic parameters*

There was a slight decrease in the average P digested, retained and excreted as the variation amongst the genetic parameters of the pigs increased; this was associated with an expected increase in the associated CVs, see **Table 5.5**. The differences between no variation and 'low' genetic variation in the above outputs were within the 0.5% standard error limits resulting from the Monte-Carlo simulations. However, the decreases in the values of the outputs reflected the fact that as genetic variation increased, a larger number of pigs were unable to meet their digP requirements, which targeted the 'average' pig for a longer period of time, and this adversely affected P retention and ultimately their growth (Chapter 4), as explained above.

The addition of variation due to feed to the pig genetic variation decreased further the average P digested, retained and excreted as the variation amongst the genetic parameters of the pigs increased. The above decreases were higher in the 'co-product' as opposed to the 'conventional' based feeds. For example, the realistic scenario that included variation due to the feed and 'normal' genotype variation resulted in 4.0 and 7.2% less P retained in comparison to the control scenario with no variation, for the 'conventional' and 'co-product' based feeds, respectively.

Increasing the genetic variation in the pig population resulted in a higher percentage of pigs meeting their requirements at the earlier stages, but a lower percentage of pigs meeting their digP requirements at the latter stages of each of the growing and finishing phases; overall fewer pigs in the population reached their requirements throughout the feed phase, see **Figure 5.5 -5.6**. Ninety-five and 82% of the pigs met their digP requirements during the finisher period, when the variation within the population was 'low' and 'normal', respectively. The combination of both variation in the feed (due to ingredient composition and mixing) and genetic variation resulted in an even lower percentage of population meeting their digP requirements. This was more profound when a 'co-product' based feed was used.

**Table 5.5** The effect of variation in ingredient composition and mixing (with (yes) or without variation (no)), of a 'conventional' and a 'co-product' - based feed, and of different degrees of variation in pig genetic variables (no variation, 'low' and 'normal' variation) on the mean and coefficient of variation (CV) of P digested, excreted and retained. The results are the outcomes of 500 simulations.

| Treatment | | P absorbed into the blood-stream, g/day | | | | P retained, g/day | | | | P excreted, g/day | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Genotype variation | Variation in ingredient composition and efficient mixer | 'Conventional' feed | | 'Co-product' feed | | 'Conventional' feed | | 'Co-product' feed | | 'Conventional' feed | | 'Co-product' feed | |
| | | Mean | CV, % | Mean | CV, % | Mean | CV,% | Mean | CV, % | Mean | CV, % | Mean | CV, % |
| No | No | 7.07 | 0 | 7.07 | 0 | 5.82 | 0 | 5.83 | 0 | 7.44 | 0 | 7.45 | 0 |
| No | Yes | 6.97 | 5.36 | 6.86 | 10.1 | 5.75 | 3.61 | 5.62 | 7.37 | 7.37 | 6.28 | 7.29 | 10.3 |
| Low | No | 7.07 | 10.9 | 7.07 | 10.9 | 5.82 | 10.5 | 5.79 | 10.5 | 7.47 | 11.8 | 7.49 | 11.9 |
| Low | Yes | 6.98 | 12.1 | 6.74 | 15.0 | 5.72 | 11.2 | 5.54 | 13.5 | 7.39 | 13.2 | 7.14 | 16.3 |
| Normal | No | 7.00 | 21.8 | 6.98 | 22.1 | 5.67 | 21.2 | 5.65 | 21.4 | 7.45 | 23.3 | 7.42 | 23.8 |
| Normal | Yes | 6.90 | 22.6 | 6.68 | 24.5 | 5.59 | 21.6 | 5.41 | 23.1 | 7.39 | 24.1 | 7.16 | 26.3 |

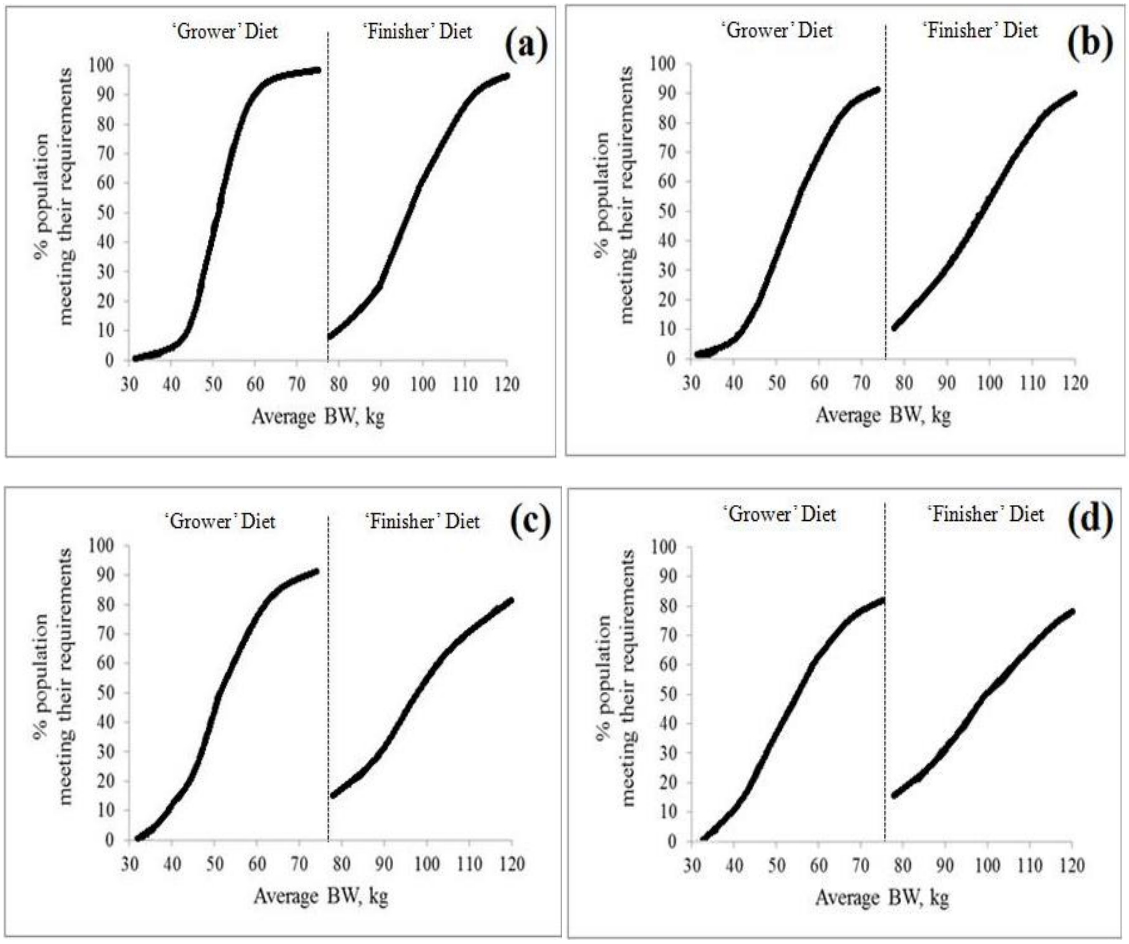


**Figure 5.5** The percentage of the population of 500 pigs that met their digestible P requirements over the average BW range 30 to 120 kg, whilst fed a 'conventional' based feed. The pigs differed in the variation of their genetic parameters (low or normal variation) and were given access to feeds that included variation in composition due to ingredient variation and mixing (with variation or no variation). The four combinations were: **(a)** pigs that included low genetic variation given access to a feed with no variation; **(b)** pigs that included low variation given access to a feed with variation; **(c)** pigs that included normal genetic variation given access to a feed with no variation; **(d)** pigs that included normal variation given access to a feed with variation.

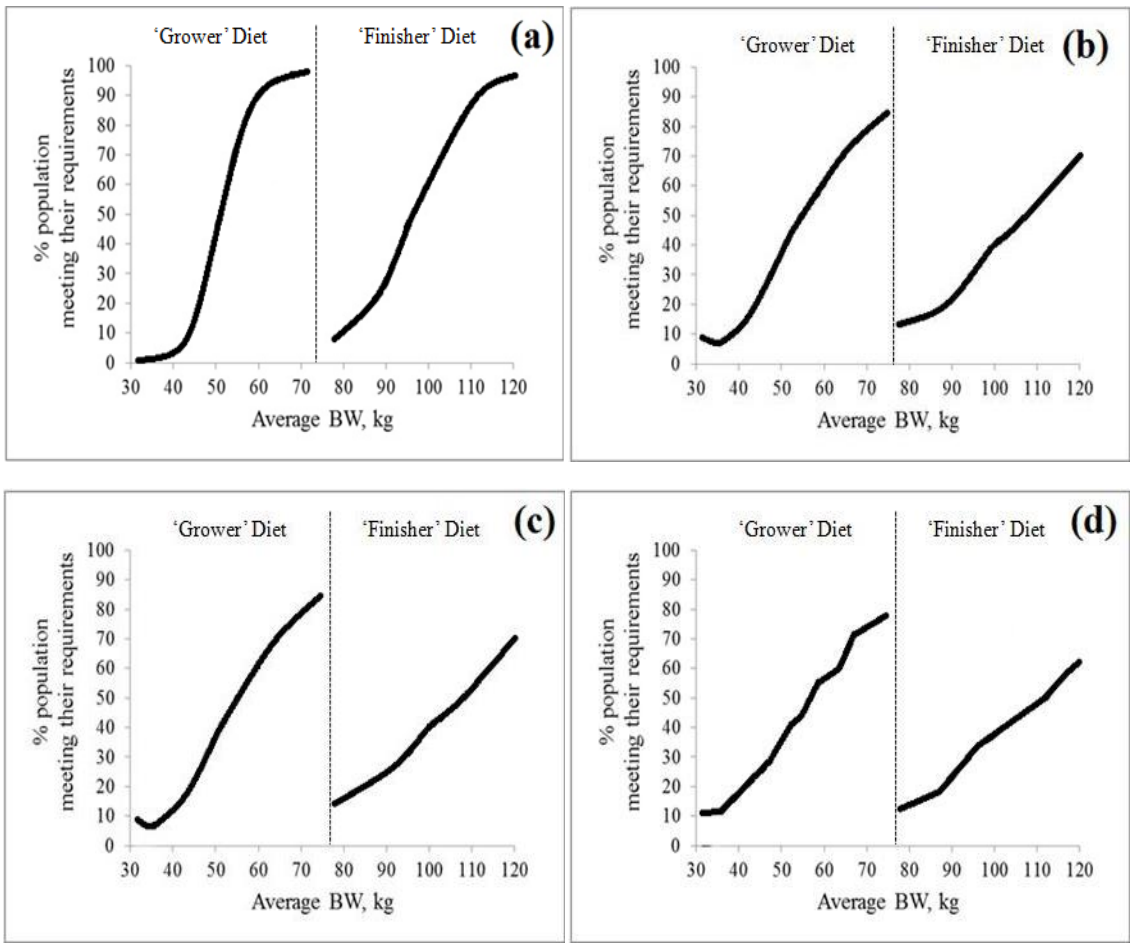


**Figure 5.6** The percentage of the population of 500 pigs that met their digestible P requirements over the average BW range 30 to 120 kg, whilst fed a 'co-product' based feed. The pigs differed in the variation of their genetic parameters (low or normal variation) and were given access to feeds that included variation in composition due to ingredient variation and mixing (with variation or no variation). The four combinations were: **(a)** pigs that included low genetic variation given access to a feed with no variation; **(b)** pigs that included low variation given access to a feed with variation; **(c)** pigs that included normal genetic variation given access to a feed with no variation; **(d)** pigs that included normal variation given access to a feed with variation.

For example, the realistic scenario variation due to the feed and 'normal' genotype variation resulted in 79 and 63% of the pigs meeting their digP requirements during the finisher period, in the 'conventional' and 'co-product' based feeds, respectively. This was the outcome of having several pigs with digP requirements well above those of the 'average' pig, were given access to feeds of low P content due to variation in ingredient composition and inefficient feed mixing.

## 5.5 Discussion

The objective of this paper was to develop a methodology that would allow us to investigate (a) how uncertainty about feed composition arising from variability in feed ingredient nutrient (P) content and mixing would affect the outputs of a nutrient utilization simulation model, and; (b) how such uncertainty would interact with the uncertainty that arises from the genetic traits of individual pigs within a population. We used a P intake and utilization simulation model as a case in point, because this was the most complete model of its kind in our disposal and because the model already included variation in the genetic characteristics of the pigs (Chapter 4). As far as we know, this is the first attempt to introduce uncertainty about feeding environment in a pig model. The simulations show that the effects of such uncertainty can be profound on model outputs, such as individuals pig performance, the variation between individual within a cohort of pigs, and the number of pigs that are either overfed or underfed P. Although the paper investigated the consequences of sources of uncertainty on P digestion, retention and excretion, we expect that the same principles would apply when dealing with the fate of any other nutrient whose intake and utilization can be subject to similar uncertainty.

The development of a stochastic model to account for uncertainty in nutrient intake and utilisation has given rise to a number of methodological challenges, namely how to generate the variation in both feed composition and pigs, and how to account for correlation between ingredients when modelling the uncertainty associated with mixing efficiency. When modelling the uncertainty associated with the mixing effect, the variation of the feed ingredients follows the modelling approach based on Bayes Theorem for conditional probabilities. It is the probability of the proportion of one

ingredient in the feed is dependent on the proportion of other ingredients. For complex feed compositions, it is difficult to formulate probability functions to describe these interactions, and therefore we developed a Monte Carlo sampling method which automatically generates sample feeds with realistic proportions of each ingredient. The model 'mixing compartment', took into account the variation of feed ingredients in energy, lysine, P, Ca contents and microbial and plant phytase activities. The study did not take into account variation in any amino acids, other than lysine, because it is generally accepted that lysine is normally the first limiting amino acid (Wellock et al., 2003). Nevertheless, the model is flexible to include uncertainty in any other amino-acid that could be first limiting in the feed, e.g. methionine. When introducing uncertainty in the ingredient composition, the model took into account the variation of feed ingredients in P, Ca, microbial and plant phytase activities only. Clearly feed ingredients differ in other nutrient contents, such as energy or amino acid content. Provided that such variation is known, the model is capable of including it in its simulations. This would not impose significant computational requirements.

The feed manufacturing industry recognizes the potential consequences of uncertainty associated with feed ingredient composition (Ru et al., 2003) and tries to account for this in various ways in their feed matrices (Sauvant et al., 2004). Nevertheless, there are some ingredients that are associated with high inherent variability, such as the co-products DDGS and potato protein (Stein et al., 2009; Pastuszewska et al., 2009), and this may lead to the results generated here: feeding on co-product based feeds led consistently to higher variation in the performance of pigs, which were assumed to be identical in their genetic growth characteristics. The feeding method used (two phase-feeding) targeted the digP requirements of the average pig at the mid-point of the weight range of the feeding phase. The outcome of the introduction of uncertainty was that a small number of pigs were oversupplied with P at the early stages and a substantial number of pigs were undersupplied with P at the latter stages of the feeding phase, for the reasons explained in the Results. Feeding the co-product based feed exaggerated the latter, and as a consequence a substantial number of pigs failed to meet their digP requirements, even by the end of the feeding phase, and as a consequence they underperformed. The model assumes that when animals do not meet their P requirements, then their performance would be penalised. This is a direct consequence of the underlying assumption of the model that there is an unviolated isometric

relationship between P and body protein, as found in several experiments (Rymarz et al., 1982; Jongbloed, 1987; Hendriks and Moughan, 1993; Mahan and Shields, 1998). However, there are suggestions that under certain nutritional conditions this relationship between P and protein may not be valid. NRC (2012), for example have suggested that pig diets can be 10% below the P requirements without any 'negative' consequences on pig daily gain. This has been explained by the existence of P 'reserves', which may exist in the body and can be used at times of P scarcity. If this is the case then the model would have overestimated some of the consequences of the variation in feed composition on performance investigated here.

Even when the uncertainty associated with the nutrient content of feed ingredients is accounted for, uncertainty in feed composition can arise from the efficiency of the mixing process. There are several factors that can affect this; they include the mixing time (insufficient or protracted mixing times can lead to ingredient segregation), feed mixer maintenance, overfilled mixer due to the bulkiness of some ingredient(s), etc (Reese and Brumm, 1992; Patience et al., 1995; Simpson, 2000). In this paper we used Monte Carlo iterations to investigate the consequences of two mixing efficiencies: one that resulted in 10% CV in the content of limestone in the feed, which is the industry accepted level resulting from efficient mixing (McCoy et al., 1994), and another that resulted in 20% CV, which is within the realistic bounds of mixing of pig feeds (Herman and Behnke, 1994). Again the effects of the mixing efficiency on the model outputs were higher in the ‘co-product’ based feeds, mainly because they contained a larger number of ingredients. A larger number of ingredients meant a smaller contribution of each ingredient in each the feed, therefore resulting in greater cumulative uncertainty in the resulting feeds and hence their outcomes. Future extension of this model could include, for example prediction of this mixing effect in the P excretion from a population, by using scenarios such as : 1) different types of feed mixers (i.e. vertical, horizontal and drum); 2) different mixing times and; 3) the effect of premixing low inclusion ingredients (i.e. MCP), rather than empirically investigating the mixing effect. Therefore, a protocol could be derived to maximize P retention and minimize its excretion from a pig population.

The consequences of mixing efficiency on the performance of a population are slightly at odds with what has been suggested in the literature. Groesbeck et al. (2007) and Traylor et al. (1994) have concluded that a CV of salt of up to 12% and 20%, respectively is adequate for maximum growth performance of pigs. One of the contributors to the differences between the literature and what has been simulated may lie in the assumptions made in the regulation of the FI of pigs. Whilst it was assumed that a pig is able to eat for the energy or the protein content of the feed (Kyriazakis et al., 1990; Emmans and Kyriazakis, 2001), here it was assumed that the same does not apply when the P content of the feed is low (Pomar et al., 2006; Lopes et al., 2009). As a result of uncertainty due to the mixing efficiency, the feed could vary in its energy and protein content, as well as P, if for example more soya was used than intended at the expense of wheat. This would have effects on the feed intake of the pigs and hence their performance.

As indicated previously, total uncertainly due to variation in feed ingredient composition and due to mixing efficiency was not the cumulative of the two uncertainties. This is because of the Monte-Carlo sampling methodology, as some of the variation caused by one process is negated by the variation caused by the other (Weiss, 2004). Due to the assumptions made by the model in the scenarios investigated, uncertainty in feed ingredient composition for the 'co-product' based feed contributed more to performance variation than uncertainty due to the mixing efficiency. This in part justifies the approach taken by feed manufacturers to use a higher number of feed ingredients in order to avoid or reduce the effects of uncertainty arising from over-reliance on a few ingredients with large inherent variation. This reduction will only in part be offset by the potential contribution of the several ingredients to the mixing inefficiency.

Previous authors have investigated the effects of genetic variation in the growth pattern of the pigs on the performance of a population of pigs (e.g. Ferguson et al., 1997; Knap, 2000, Pomar et al., 2003; Wellock et al., 2004; Sandberg et al., 2006). Like Pomar et al., (2003) we investigated the effects of the decrease in the variation of the genetic parameters, from the current estimated CV associated with these traits. This is because for some time producers desire a reduction in the variability within a batch, as

this is associated with financial consequences (Patience et al., 2002; Huang and Miller, 2004; Patience and Beaulieau, 2006; Tokach et al., 2007). The outcomes of the simulations suggest that such a reduction in variability in performance can indeed be achieved through a reduction in the variation of the genetic growth parameters. This was accompanied by a higher number of pigs achieving their digP requirements over each feeding period. Current advances in molecular genetics now allow breeding companies to evaluate more precisely and control genetic variability in commercial populations (Sullivan, 2007). The simulations in this paper quantify the benefits that can arise from this.

The paper also investigated the interactions between uncertainty due to variability in the feed and due to variability in the pig. The most striking outcome of the simulation was the fact that in the presence of uncertainty in the feed composition, the number of pigs that met their digP requirements was similar, irrespective whether the variability in the pig genetic parameters was high (normal) or low. This was especially the case when pigs were fed on a co-product based feed, where the percentage of pigs that met their requirements at the end of the finisher period was ~60%. In other words, when pigs were simulated under conditions likely to be encountered in commercial environments, it was uncertainty about the 'co-product' based feed composition, rather than pig genetic characteristics that shown to have the dominant influence on variability in pig performance. Currently there is an increased interest in how to deal with variability within a batch of pigs (Patience et al., 2002; Patience and Beaulieau, 2006), due to the financial consequences associated with it, and feeding strategies to overcome this are being developed (Douglas et al., 2014a, b). The model developed here is able to account for the interactions between feeding strategies and variability within a batch of pigs. It can be envisaged that such interactions may arise if the smaller pigs are given access to a different feeding regime, associated with these uncertainties.

## 5.6 Conclusion

We have developed a methodology that is able to account for uncertainty due to variation in feed composition and pig genotype. The methodology has pointed towards

some issues that need to be addressed to increase model accuracy and utility. Such issues to improve the model accuracy are to take into account the inherent variability in the ingredient energy and lysine concentrations, as well as the development of a ‘bone growth compartment’, which can be utilized at times of dietary P deficiencies. The methodology has demonstrated the potential of uncertainties to affect the predictions of a nutrient intake and utilization model. The developed framework can be used to investigate the consequences for pig performance of uncertainty as regards several components of the system, namely the pig, its feed and its environment, on pig performance and the uncertainty associated with it. Such consequences are likely to have significant impact on decisions about how to feed pig populations that are subject to uncertainties.

# Chapter 6. General Discussion

## 6.1 Introduction

Minimizing the environmental impact of pig production without jeopardizing performance is a priority in order to achieve sustainable pig production. Traditionally, spreadsheets have been used to assist advisors and nutritionists in making optimal cost decisions, i.e. least cost formulations. Recently, mathematical models have also been used to minimize the environmental impacts of pig production by maximizing the efficiency of nutrient utilization, and hence minimizing the cost of production (van Milgen et al., 2008; Pomar et al., 2011; Pomar and Pomar, 2012; Moraes and Fadel, 2014). There are several benefits that may arise from their use, as several factors that affect the production system and their interactions may be considered simultaneously.

Phosphorus (**P**) is an important mineral for both the metabolism and skeletal development of the growing pig (NRC, 2012). In pig feeds, P is the third most expensive nutrient required, after carbohydrates (energy) and protein. On the other hand, P is responsible for the environmental eutrophication that results from pig production systems, contributing around 14% of the total diffuse P load from livestock to UK waters (White and Hammond, 2006). The application of mathematical models of P intake, digestion, utilisation and excretion integrate our knowledge on the: (1) forms of P available in the feed; (2) the biological processes taking place in the gastrointestinal tract regarding the digestion of P (**digP**) into the bloodstream;(3) the utilisation of digP in the body and; (4) ultimately its excretion in the environment. In addition mathematical models can be valuable tools for estimating pig individual requirements for digP both for the 'average' pig and the individual within a population, and digP derived from feeds in each unique farm production scenario. Thus models can have an important role in providing effective feeding management that can be used in the decision-making process to enhance the feeding system, whilst minimising its environmental impact (Tedeschi et al., 2004).

The objective of this chapter is to discuss the progress achieved in developing a P model of intake, digestion, utilisation and excretion and the issues that arise from it. The following discussion firstly addresses some of the influences of methodology and possible future improvements on: (1) modelling the fate of dietary P in the gastrointestinal tract and; (2) modelling the utilization of digP in the bloodstream. The application of the stochastic model to different feeding management regimes is then discussed for their impact in P utilization and implications this has for the industry. The feeding management regimes considered were: (1) different feeding phases throughout the feeding period of growing and finishing pigs (phase feeding); (2) sorting pigs into a light and a heavy group and feeding each group according to the requirements of their group average BW; and (3) dietary manipulations including different levels of microbial and plant phytase and dietary Ca. New avenues for future research are considered, in order to be able to create a more mechanistic model that will also be able to explore different and more sophisticated feeding management techniques to maximise the dietary P utilisation and minimize its excretion to the environment. The general discussion concludes with a brief overview of the major findings of this thesis.

## 6.2 Methodological considerations for model synthesis

### *6.2.1 Modelling the fate of dietary P in the gastrointestinal tract*

#### *6.2.1.1 Factors affecting P digestion in the stomach*

In contrast to the feed-tables of NRC (2012) which report P in each feed ingredient in terms of standardized total tract digestibility, the model developed in this thesis followed the approach used by van der Klis and Versteegh (1996), Dias et al.(2006) and Létourneau-Montminy et al. (2011), in which the total P of each ingredient composing a feed was divided into the easily digestible phosphate P (**NPP**) and the phytate P (**oP**). An advantage of using this method is the creation of a more mechanistic approach to P digestion, because it takes into account dietary factors that affect the P digestibility such as the activity of exogenous and endogenous phytase enzymes and dietary Ca concentration. According to this approach there is no unique value of digP content for a

feed ingredient, as this would depend on feed composition. This clearly presents a challenge to the industry, which wishes to have estimates of such a value.

The management of pig production requires the model to examine the factors affecting the P absorption into the bloodstream, namely, phytase enzymes and dietary Ca concentration. Since the majority of P, 60-80%, in 'conventional' feeds is made up of oP (Kornegay, 2001), it is crucial that the model is able to simulate the oP dephosphorylation by phytase enzymes into easily digestible phosphate. Because the oP dephosphorylation is not a linear function of phytase activity (van Milgen et al., 2008), a first-order kinetic exponential equation was used to quantify the curvilinear degradation of oP. This model used exponential equations from *in vitro* and *in vivo* experiments to quantify the effect of oP dephosphorylation by microbial and plant phytase enzymes, respectively, while others, such as Létourneau-Montminy et al. (2011), have used more sophisticated Michaelis-Menten kinetics based on *in vivo* experiments to account for such relationships. The only limitation of using an exponential equation for the plant and microbial phytase activity was that it considers that the feeds will always have sufficient oP to act as a substrate to phytase, which is a realistic assumption. An improvement to the current methodology for estimating the oP desphosphorylation by phytase enzymes would be to progressively replace total oP by 'reactive' and 'non-reactive' oP, something that no model has yet been able to take into account due to the lack of data for many ingredients. This is because not all oP is soluble and susceptible to dephosphorylation by phytase enzymes; for example rice bran and corn gluten meal have 85-92 % and 28-48 % oP susceptibility, respectively (dos Santos and Bedford, 2012). Through model evaluation (Chapter 2), it became clear that there is a difference in the oP dephosphorylation from different ingredients using the same phytase activity (Leske and Coon, 1999; Adeola et al., 2004); the reasons for this have been presented in the same chapter. Since the majority of P is in the oP form, a better understanding of oP dephosphorylation can lower the need for supplementation of expensive inorganic P, increase the P utilization and decrease the P excretion.

The model considered whether the feed had been pelleted; if this was the case it was assumed that there was a 50% reduction in phytase activity (Jongbloed andKemme, 1990; Jondreville and Dourmad, 2005). Nevertheless, new strains of microbial phytase

are now more resistant to thermal denaturation, and this has not been taken into account by the model. In addition, in the model the microbial and plant phytase were considered to be only active in the stomach, where the acidic pH favoured phytase activity, because of the high solubility of the oP (Kiarie and Nyachoti, 2010) and because the protease enzymes are not so active (Zhao et al., 2010) in comparison to the small intestine (**SI**). Nevertheless, new strains of microbial phytase have been produced that are protease resistant and have wider range of optima pH (Quan et al., 2004). In theory they could be partially active even in the alkaline environment of the SI, something that the model has not considered, and thus the model might be under-estimating the P digested. These new strains can only be partially active in the SI because of the prominence of the formation of insoluble and indigestible Ca-oP complexes (Selle and Ravindran, 2008). In theory the use of thermo-stable and protease resistant microbial phytase, in combination with acidifiers that decrease the pH of the gastro-intestinal tract, can help oP be soluble for a longer time so that more dephosphorylation might take place with the same amount of phytase activity, but this hypothesis has not been tested. However, the model is capable of accounting for such advances, provided that relevant data for their effects are available in the literature.

Finally, excessive supplementation of calcium carbonate increases the pH in the gastrointestinal tract and the higher availability of Ca substrate increases the formation of Ca-oP complexes (Selle and Ravindran, 2008), which in turn lowers the oP dephosphorylation. Therefore, a possible improvement in the approach of the current model could be the quantification of the pH fluctuations in the stomach due to the supplementation of different levels of calcium carbonate and its effect in the oP dephosphorylation. This is something that the model ignores, due to the large number of extra parameters that would need to be quantified, as well as the complexity of the stomach's rapid pH fluctuations (Pontoppidan et al., 2007).

#### *6.2.1.2 Factors affecting P digestion in the small intestine*

The model is able to simulate the effect that high dietary Ca from supplemental calcium carbonate has on the oP dephosphorylation by the endogenous SI phytase enzymes

(Chapter 2), even though the data used to derive the relationship were from a broiler study (Plumstead et al., 2008). It is appreciated that such data could be generated in pigs and may alter the function of the relationship. Nevertheless the sensitivity analysis conducted (Chapter 3) suggested that the endogenous phytase in the SI does not significantly affect model outputs.

The absorbability of phosphate from the SI into the bloodstream is not regulated as strictly as Ca (Veum, 2010; France et al., 2010). The P absorption into the bloodstream under normal dietary conditions has a constant digestibility coefficient (Ekpe et al., 2002; Schulin-Zeuthen et al., 2007; Lopes et al., 2009; Létourneau-Montminy et al., 2012). Nevertheless, at very low dietary P content the digestibility coefficient increases because of an active transport taking place, rather than passive transport (Breves and Schroder, 1991; Fernandez, 1995). The model did not take into account the active transport of P, because feeds with such low dietary P are rare in realistic scenarios. In the model, the digestibility coefficient for P was assumed not to vary quantifiably between individuals of different genotypes or ages, in accordance with Kyriazakis (2011). The latter suggestion implies that the digestive system of the pig operates at an optimal capacity, perhaps due to natural selection. However, there are conflicting suggestions in the literature regarding this; Kemme et al. (1997) and Letourneau-Montminy et al. (2012) have suggested that the P digestibility of the same food is higher in growing than in finishing pigs, whereas Jongbloed (1987) suggested an increasing P digestibility as body weight (and hence age) increased. Based on the sensitivity analysis conducted in Chapter 2, the digestibility coefficient has a significant effect on model outputs. For this reason it would be valuable if this issue could be clarified with appropriately designed experiments (i.e. through isotope dilution techniques). The SI is the only part of the gastrointestinal tract where phosphate digestibility into the bloodstream takes place (Veum, 2010).

Because P is a costly mineral, and has high potential environmental impact, its utilisation has been studied more extensively than most other minerals, such as Ca (Soares, 1995), despite the fact that Ca negatively influences P digestion (Selle et al., 2011). While the model simulated the effect that Ca had in the SI and the formation of Ca-oP complexes, the model was not able to simulate the antagonistic effect that Ca had

on the exogenous phytase in the stomach due to "little tangible evidence" (Selle et al, 2009). The effect of dietary Ca in the stomach is likely not to be very important, because the acidic environment of the stomach favours oP dephosphorylation in comparison to the formation of Ca-oP complexes, unless there is an excessive supplementation of calcium carbonate. This, in turn, would increase stomach pH and therefore decrease the solubility of oP and increase oP-Ca complex formation.

A significant limitation of the developed model relates to its inability to simulate Ca digestibility (**digCa**) in the same way as P digestion. Currently there is no model that can accurately predict the digCa, other than rough estimations, with Létourneau-Montminy et al. (2011) having made an initial modelling attempt towards this. They suggested that the apparent digCa coefficient has a value of 0.55, irrespective of the dietary vitamin D content. However, vitamin D increases the digestibility coefficient value of Ca (Kornegay, 2001; O'Doherty et al., 2010) and should be taken into account when simulating digCa. Phytase enzymes can also indirectly increase digCa, because less oP will be available for the formation of indigestible Ca-oP complexes. To be able to determine standard total tract digestibility values for Ca it is necessary to determine basal endogenous losses of Ca, something that no author has ever reported (González-Vega and Stein, 2014). Recently, attempts have been made to determine the apparent total tract digCa for a limited number of ingredients (Bohlke et al., 2005; Stein et al., 2011; González-Vega et al., 2013; Sulabo and Stein, 2013), but more experimentation needs to take place before serious Ca modelling attempts can progress.

#### *6.2.1.3 Factors affecting P digestion in the large intestine*

Although phosphate is transported across the mucosal apical membrane in the large intestine (**LI**), this process is likely to be limited to the maintainance of local mucosa tissue growth and metabolism (Shen, 2006). It was thus deemed not necessary to quantify in the model the phosphate which was utilized by the LI. Despite the insignificant contribution of LI to P retention in the pig (Liu et al., 2000; Fan et al. 2001; Shen 2006), a significant proportion of oP which was not dephosphorylated in the

stomach and the SI is hydrolysed by the LI's endogenous micro-flora phytase (Schlemmer et al., 2001). Similar to the oP dephosphorylation in the SI, the model is able to simulate the high dietary Ca from supplemental calcium carbonate which increases colonic pH, and results in the reduced degradation of oP in the colon, based on the study of Sandberg et al. (1993). Due to the importance of LI processes in determining the excretion of soluble P, which is the main reason for eutrophication (Bannink et al., 2010), more studies are needed to repeat the methodology of Sandberg et al.(1993) and examine the impact of breed and stages of growth on this process.

### *6.2.2 Modelling the inevitable P losses*

A first determinant of P requirement is the inevitable digP losses (Pfeffer et al., 2005; Bannink et al., 2010). The model assumes that the P absorbed from the gastrointestinal lumen into the bloodstream, is prioritised to replenish the endogenous P losses and has a 100% efficiency utilisation, in accordance to Rodehutscord et al. (1998).This is also consistent with the estimate of the efficiency of utilisation of other nutrients for maintenance (Sandberg et al., 2005). The determination of the total endogenous P excretion into the gastro-intestinal tract originating from saliva, gastric and biliary juice and other P sources is a major challenge because dietary and endogenous P is mixed completely in the gut, and P digestion occurs without differentiation between endogenous and dietary origin (Fan et al., 2001; France et al., 2010). Some of this P of endogenous origin can be reabsorbed, but the remainder appears in faeces. Due to these difficulties, the model considered the endogenous P losses from P found in digesta or faeces of pigs fed P-free feeds, which is in accordance with France et al. (2010) and Létourneau-Montminy et al. (2011). An innovative aspect of the model in this thesis is the quantification of the maintenance P requirement as a function of body protein (Chapter 2) rather than a function of body weight which has been used previously by Jongbloed (1987), Rodehutscord et al. (1998), Lopes et al. (1999) and Létourneau-Montminy et al. (2011).

Some authors (Shen et al., 2002; Ajakaiye et al., 2003; Petersen and Stein, 2006; Pettey et al., 2006; NRC 2012) have estimated the total endogenous P secreted in the gut

through a regression method. The regression technique is a linear relationship between graded levels of P in the feed and the P output in faeces (González-Vega and Stein, 2014). Total endogenous losses of P can be calculated as the y-intercept of the linear regression after extrapolation back to zero input of P (Fan et al., 2001; Kil et al., 2010). The total endogenous P loss is a function of food intake (**FI**), body weight and dietary fibre content (Nyachoti et al., 1996).

Total endogenous P secretion originates from the gastric juices (Fan et al. 2001), thus FI intake pre-determines the release of the digestive juices to aid digestion. Total endogenous P losses could be affected by the food composition, with a low energy feed causing the pig to increase FI to try to compensate (van Milgen et al., 2008; Letourneau-Montminy et al., 2011), causing more release of digestive juices to aid the digestion of this FI, thus increasing the maintenance P requirements. The main problem of expressing total endogenous P losses as a function of FI in modelling is that FI should not be used as an input. It is preferable if FI is an output of the model, as this enhances model relevance. Fixing the FI at a certain level greatly reduces the model flexibility in finding combinations that meet animal digP requirements at a minimum cost (Moraes and Fadel, 2014). In addition, differences in housing, genotype, bulkiness, energy and Pr content of the feed impact on FI (Wellock et al., 2013).

### *6.2.3 Modelling the utilization of digestible P in the bloodstream*

This section discusses the modelling synthesis of the post-digestive P utilization, which is also the regulatory part of the model that depends on the extent to which actual supply of digP meets the requirements. Probably the most important prerequisite for successful feeding management which minimizes the excretion of P and the environmental impact of growing pigs is the estimation of the P requirements of each individual.

A pig in this model was described through the following genetic traits: (1) protein at maturity (**$Pr_m$**); (2) lipid to protein ratio at maturity (**$LPr_m$**);and (3) scaled maturing rate (**B***) in accordance with Ferguson et al. (1997), Knap (2000), Pomar et al. (2003) and

Wellock et al. (2004). The main challenge of using these genetic traits was that, in order to estimate their values, experiments are usually needed where pigs are grown to maturity and serial slaughter is applied at different BW (Ferguson and Gous, 1993). Nevertheless, by using an alternative technique called 'inverted modelling' or 'reverse simulation' (Knap et al., 2003; Doeschl-Wilson et al., 2005; 2007) it became easier and cheaper for genetic parameters to be estimated. The model is 'inverted' in the sense that the conventional model input traits (the underlying biological traits) are treated as model outputs that need to be determined through the inversion process, and the parameters of the conventional model output traits are treated as known inputs (Doeschl-Wilson et al., 2007). One methodology of 'inverted simulation' works by comparing observed data (i.e. ADG) with model outputs and adjusting model parameters so that predictions corresponded (as closely as possible) to observations. Another more elegant methodology is through algebraically rework the model equations, to end up with the fundamental parameters (rather than the observations) on the left-hand side, and coding this inverted model as a new computer program (Knap et al., 2003).

While this model used three genetic traits to describe a pig, Brossard et al. (2009) and Vautier et al. (2013) used five genetic traits, to achieve this. Two of their genetic trait descriptors related to FI but, as explained above, FI should preferably not be a model input (Wellock et al., 2003). Nevertheless, the main advantage of the methodology used by Brossard et al. (2009) and Vautier et al. (2013) was that the pigs did not need to grow to maturity to derive their genetic parameters, and the pig growth parameters are much more easily derived by breeding companies and thus can be more attractive for use in genetic selection programmes.

The model estimated the requirements based on the sum of requirements for maintenance and growth, as well as taking into account the efficiency of digP utilisation for growth ($\mathbf{e_{growth}}$) (Chapter 2). The $e_{growth}$coefficient was calculated from the slope of the regression of P retention (g/day) against digP intake (g/day) (Pettey et al., 2006). The experiments used in this study to derive the $e_{growth}$ were with semi-purified feeds (Rodehutscord et al., 1999; Pattey et al., 2006), with the source of dietary P being mono-sodium or mono-calcium phosphate, which is almost completely digestible according to Rodehutscord et al. (1994).Therefore, there is digestion of most of the P

intake and it is only left to net efficiency of utilisation to retain the P, provided that the P requirements were not exceeded. A more accurate technique to estimate this would be through the use of a P isotope dilution, in which one can trace exactly the ratio of radioactive tracer $^{32}P$ which is digested and the ratio of the digP retained into the body. Pattey et al. (2006) concluded that $e_{growth}$ declined with pig size, in contrast with the suggestion of Kemme et al. (1997). The $e_{growth}$ at 27 kg BW of Pattey et al. (2006) is in agreement with Rodehutscord et al. (1999) and NRC (2012), which stated the $e_{growth}$ to be 0.94, and this is the coefficient adopted in this model. No variation in $e_{growth}$was considered between stages of growth, sex or genotypes, even though the model sensitivity analysis clearly showed that variation in $e_{growth}$ will have a significant effect on model outputs. This is in accordance with Kyriazakis (2011), who stated that there is no measurable genetic variation in nutrient utilisation between animals. Significant effort has been put into the consideration of whether the efficiency of individual amino acid utilisation is a constant or a variable (Sandberg et al., 2005). The importance of this parameter in model outputs justifies further effort being directed toward this issue.

### 6.3 The effect of Ca in P retention

It was discussed in the previous section that the main limitation of this model was the inability to simulate the digCa, because only a limited number of ingredients have been assessed for their apparent digCa and no study ever investigated endogenous Ca excretion (González-Vega and Stein, 2014). Another gap in the knowledge, which makes it very difficult to accurately simulate the fate of dietary Ca, is the unknown efficiency of digCa utilisation for growth, because no study has ever attempted to quantify this important parameter. Ca requirements are estimated directly from digP requirements using simple ratios (de Lange, 2013).

Despite Ca having a negative effect on P digestion, it is vital for P retention into bone, because there has to be a 2:1 ratio between digCa and digP (Létourneau-Montminy et al., 2012) and suggested overall feed ratio of digCa to digP between 1.55:1 and 1.70:1. Nevertheless, the model developed here simply assumes that enough Ca will be digested, irrespective of whether the feed is first limiting in Ca, and the digested P will always have the necessary Ca to form hydroxyapatite (bone). An improvement to the

current model could be the separation of the P retained in the body between the bone and the soft tissue. The model currently assumes that when animals do not meet their digP requirements then both P and protein retention is penalized, because there is a close relationship between the two. However, there are suggestions that, under certain nutritional conditions, the isometric relationship between P and protein may not be valid. It is possible that the bones can act as P storage which can be utilized at times of relatively small P deficiency (Henry and Norman, 1984; Hurwitz, 1996; DeLuca, 2008; NRC, 2012). NRC (2012), for example, have suggested that pig feeds can be 15% below the P requirements without any 'negative' consequences on pig daily gain, although there is inadequate information in the literature to support this. Therefore, a future development of the model would be to simulate different phases of P deficiency (e.g. an initial phase of bone weakening while maintaining performance, followed by a phase of growth  reduction) and compensatory bone mineralization when P supply exceeds the requirements (van Milgen et al., 2008).

### 6.4 Development of the stochastic model

Previous literature attempts to introduce stochasticity to nutrient utilisation models (Pomar et al., 2003; Wellock et al., 2004; Brossard et al., 2009; Vautier et al., 2013) have employed a Monte-Carlo (**MC**) methodology to simulate the effect of variation in pig genetic traits on performance. This was also the approach taken in this thesis. The novelty of this model lies in that it also dealt with uncertainty in feed composition (arising from variability in ingredient nutrient content and mixing efficiency). As far as we are aware, this is the first attempt to account for such uncertainty in a nutrient utilisation model. Nevertheless, in order to make the model fully stochastic there is the need to take into account variation in the other components of the environment, such as variation in ambient temperature or the social environment, something that this model ignored. We expect that developing the model into a fully stochastic one would be computationally achievable; other authors have modelled the effects of environmental stressors (Wellock et al., 2003; Renaudeau et al., 2010) and the uncertainty associated with social stressors (Wellock et al., 2004).

The model was developed in Excel using VBA programming language and this raised a number of issues, especially in relation to its running requirements. It would be preferable if further model development is undertaken in free packages such as "R" or "Python" that enable more flexible algorithm development and introduction of uncertainty for large datasets. "Python" is more preferable than "R" because it is a fully featured programming language, which means it can be used to create a complicated model. "R" does not offer more than minor features for interfacing with operating systems and other important programming tasks. Nevertheless, in comparison to "Python", the graphical capabilities and statistical analysis of "R" are outstanding.The main reason for choosing VBA over other programming languages such as 'R' or 'Python' is because the model would be more appealing to nutritionists which will be the end users of such a model. Nutritionists are knowledgeable and comfortable in the usage of excel in comparison to other programming languages and they would require minimal training as they would simply need to enable the macros and the VBA codes embed in the speadsheet would automatically do all the complicated calculations.

The main advantage of using a stochastic model is that it determines: (1) the percentage of the population that had their digP requirements met throughout the BW period 30 to 120 kg of the population; and (2) the percentage of the population that were seriously (+/-25%) underfed or overfed dig P in comparison to their individual requirements at any one stage of their growth. Therefore, a stochastic model can predict with more accuracy the cumulative P excretion by a group of pigs, as well as individual performance; therefore it can predict performance variability within a group. Under the assumption that digP requirements in a population of animals follow a normal distribution (Patience and Beaulieu, 2006; Moraes and Fadel, 2014), half of the pigs are being underfed and half of the pigs are being overfed P when supply is targeted to the average animal. The reasoning is that, in a normal distribution, the mean coincides with the median, which represents the $50^{th}$ distribution percentile. Nevertheless, it should be noted that in case of poor pig health, even a sub-clinical outbreak, this distribution is likely to change and can be skewed.

The INRA feed tables (Sauvant et al., 2004) have been used to input the average and standard deviation (SD) of oP and phosphate contents for each ingredient, and a MC methodology was applied to investigate the effect of variation in ingredient composition

(Chapter 4). A better understanding of the feed nutrient composition and its variability can lead to a more precise feed formulation, reducing dietary costs and excessive P excretion. An improvement of the current approach would be to introduce uncertainty in other nutrient resource contents besides P, Ca, microbial and plant phytase activities only, namely energy or amino acid contents, therefore making the simulations more realistic. Nevertheless variations in such nutrient resources currently have not been included in the INRA feed tables used in this thesis. This highlights the heuristic value of the developed model, as it identifies the requirements for inputs that would improve the value of the model outputs.

An important uncertainty associated with feed composition, which was quantified in this thesis, arises from feed mixing (Groesbeck et al., 2007). The novel methodology used to address the uncertainty due to mixing was more complicated than the methodology used for the quantification of the variability in the ingredient variation, and followed the principles of Bayesian inference (Chapter 5). The model criteria for determining the efficiency of mixing were based on simulating a feed with limestone having a fixed % CV in the resulting mix in accordance to Herrman and Behnke (1994), McCoy et al. (1994) and Groesbeck et al. (2007). Extensions of the methodology to account for the uncertainty in diet composition due to mixing efficiency should be able to account for scenarios such as: 1) the effects of mixing time (insufficient or protracted mixing times can lead to ingredient segregation); 2) the consequences of different type of mixers (i.e. vertical, horizontal and drum); 3) mixer maintenance effects; and 4) the effects of mixer overfilling due to the bulkiness of some ingredient(s) (Reese and Brumm, 1992; Patience et al., 1995; Simpson, 2000). As previously indicated, this is the first time that a nutritional simulation model has been developed to account for the sources of variation in diet composition. There is significant complexity in achieving this and perhaps this is the reason that it has not been previously addressed. The methodology developed here is the first step in effectively addressing this.

### 6.5 Practical implimentations

Phase feeding is a very effective feeding management strategy to ensure that the feed matches more closely the requirements of the animals. The model clearly demonstrates that increasing the number of feed changes (feeding phases) resulted in decreases in P excretion, and increases in P retention. As well as resulting in reduction in P excreted, increases in the number of feed changes resulted in effects on performance: increasing average daily gain (**ADG**), protein retained and decreasing food conversion ratio (**FCR**). Increasing the number of feeding phases resulted in a lower production time and a lower percentage of pigs being under-fed and over-fed at the beginning and at the end of each feed phase, respectively. The model can be developed further, to find the number of feeding phases that are most cost effective, since increasing the number of feeding phases results in an increase of cost because of the storage and handling of the extra feeds produced. The ultimate development of the model could be towards the use of feed blending in precision feeding systems, which uses the mixture between two (basal) feeds to deliver the appropriate amount of nutrients in the feed at group or individual level (Pomar et al., 2009).

The model also suggested that, although there were reductions in the P excreted when the sorting strategy was applied, these were relatively small compared to phase feeding. Sorting has been effective in improving the performance of the 'lightest' population, by supplying them with digP closer to their requirements, but at the same time resulted in an increase in the P excreted. The model has not considered the economic implications of the management strategies considered, but was developed in terms of minimisation of P excretion whilst animal performance was maximised. The development of an economic module within the model will enable such an extension.

The model confirmed the well-established notion that dietary manipulations and conditions in the lumen of the stomach and the SI affect primarily the degradation of oP by phytase enzymes (Bannink et al., 2010). A main conclusion derived from model testing (Chapter 2) was the validation of the industry recommendations of 750-1000 FTU microbial phytase supplementation. As previously discussed, the response to oP dephosphorylation by microbial phytase is curvilinear (Kornegay, 2001; Adeola et al., 2004; Jondreville and Dourmad, 2005; Kies et al., 2006), but the maximum cost-effective supplementation is at approximately 1000 FTU because the oP

dephosphorylation is almost linear to this point. When exceeding the supplementation of 1000 FTU, even though there was still oP dephosphorylation, it was at a decreasing rate up to 2500 FTU and beyond that point no more oP dephosphorylation took place (Kies et al., 2006). Therefore, it is most cost effective to supplement microbial phytase at 1000 FTU, due to diminishing returns. For a 'conventional' UK based feed, 1000 FTU microbial supplementation can dephosphorylate 36% of the total oP in the feed.

The term 'phosphorus equivalence value' is used to empirically quantify the digestible P produced by a given amount of phytase (Kornegay, 2001) and is widely used by the industry. The model shows that an equivalency value of phytase to digP is not representative and can be misleading. Normally the recommended dosage of phytase is 750 FTU/kg to deliver 0.8g digP. It should be realised, however, that the relationship between units of phytase and liberation of phosphorus is not linear, and that it also depends on the dietary oP content. Because of these relationships, the P equivalence value of phytase can be predicted to be lower or higher at this inclusion rate. It should also be pointed out that this is only true for feeds with low plant phytase. For example, the model found an equivalency value of 0.77 and 0.90 g digP for 1000 FTU/kg for 'convectional' growing and finishing feed, respectively. The difference in the equivalency value of phytase to digP between 'grower' and 'finisher' feeds clearly shows that, because the 'finisher' feed had greater oP content because it contained more barley and less soybean meal than the 'grower' feed, more oP was dephosphorylated, which resulted in a high equivalency to digP.

This thesis has developed a stochastic model approach that is able to predict the P intake, digested, retained and excreted in growing and finishing pigs in a population of different genotypes, offered access to feeds of different composition. The novelty of this model originated from the development of a methodology that allows: (1) the estimation of soluble P excretion; (2) investigation of uncertainty about feed composition (arising from variability in ingredient nutrient content and mixing efficiency) and its consequences on P utilization; (3) estimation of the requirements for maintenance and growth for pigs of different genotypes. There are some developmental improvements identified, but in general the model is the first step in representing P retention and excretion and developing feeding strategies that aim to minimise P excretion.

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