Effect of Phytase On Availability Of Phosphorus To Growing Pigs

by

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NOTTINGHAM
UNIVERSITY
AGRICULTURAL
AND FOOD

I eat, I thrive, therefore I am

Not muscl'd steer, nor virile ram

But growing pig in every sense;

In life, in limb, in soul... And hence

With snuffling snout and grateful grunt,

Forsaking all but fare in front

And that which appetite must dictate

I clear the trough, my aim; to sate.

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Abstract

Problems caused by excessive phosphate output from the animal production sector were addressed by examining means of lowering phosphate excretion of growing pigs. A review of literature looked at the requirement, dietary allowance, and availability of phosphorus to growing pigs. Variation in estimated phosphorus requirements needed to be resolved before dietary allowances could be matched to requirements. It was recognised that phytate-phosphorus present naturally in cereals would be sufficient to meet the pig's requirement for phosphorus if it could be hydrolysed prior to or during digestion. The use of *Aspergillus niger* phytase to improve phytate - phosphorus availability, and thereby lower faecal phosphate output, was explored.

A series of trials assessed the efficacy of phytase from *Aspergillus niger* (var *ficuum)* and looked at the phosphorus requirement of growing pigs. The first studied the effects of phytase on apparent ileal and total tract digestibility, and examined whether an alteration in fermentation induced by dietary inclusion of yeast (*Saccharomyces cerevisiae*) could influence phosphorus availability. Six gilts, cannulated at the terminal ileum, were used in a 3 x 3 latin square balance trial. A high-phytate barley based diet containing 4.7g phosphorus (2.3g present as phytate-phosphorus) was fed (1) as a control, (2) with 2.5g (1000 units) *Aspergillus niger* phytase/ kg or (3) with 5g *Saccharomyces Cerevisiae* yeast/ kg. For each period, a 10-day acclimatization to the diet was followed by a 5-day total faeces and urine collection period, and thereafter by a 5-day ileal sampling period. Phytase addition resulted in an increased apparent ileal digestibility of phosphorus from 0.259 to 0.387 ($p = 0.094$, s.e.d. = 0.054), an increased total tract digestibility from 0.0483 to 0.632($p = 0.074$, s.e.d. = 0.070), and a 37% reduction in faecal phosphate output ($p = 0.018$). Any alteration in fermentation as a result of yeast addition did not influence phosphorus digestibility. It was concluded that the addition of 1000 phytase units/kg feed caused hydrolysis of phytate and therefore an improved availability of phytate phosphorus.

A second balance trial looked at the relationship between level of dietary phytase and phosphorus digestibility, in order to derive an optimum inclusion rate. 12 gilts of

25kg liveweight were used in a triplicated 4 x 4 latin square trial. Treatments were a maize-soya basal diet (1) containing 5g/kg phosphorus (of which half was present as phytate phosphorus) to which was added phytase at levels of 500 (2), 1000 (3), and 1500 units phytase/kg (4). Calcium to phosphorus ratio was 1.5:1. Each 10 day acclimatization period was followed by a 5 day total faeces and urine collection. Addition of phytase increased apparent phosphorus digestibility (over the control) at all levels of inclusion ($p = 0.05$, s.e.d. = 0.025). The quadratic response of phosphorus digestibility to dietary phytase was described by the equation $y = 0.5832 + 0.000162x - 0.84E^{-7}x^2$ ($p = 0.048$; y = apparent phosphorus digestibility, x= phytase units/kg feed). The maximum response was calculated at 1000 units/kg, however, the shape of the curve indicated that 400-500 units/kg was the economic optimum.

Having established repeatable increases in phosphorus digestibility with phytase, two growth trials were used to assess the amount of phytate-phosphorus that could become available through use of the enzyme. In both trials, graded levels of non-phytate phosphorus were achieved by addition of inorganic phosphate to a low phosphorus basal diet. Each phosphorus level was fed *ad libitum* either with or without phytase added at 1000 units/kg feed. The first trial looked at young pigs growing from 10 to 25kg liveweight. Growth performance, whole body mineral content, bone strength and bone mineral content were used as response criteria. 72 individually penned piglets weighing 10kg were assigned randomly within sex to 2 (basal diet), 2.5, 3, 3.5, 4, 4.5, 5, 5.5 or 6 g non-phytate phosphorus/kg at a constant calcium level (8 g/kg). At 25kg the pigs were killed, and the right femur and right third and fourth metatarsal bones removed for breaking force determination using an Instron detector. Samples of the ground whole body were analyzed for phosphorus content.

Reduced daily gain of pigs receiving the basal (2g/kg) diet was counteracted by the addition of phytase, approaching that of pigs on the 3.5 g/kg diet ($p= 0.085$, sed = 0.056). Femur strength and phosphorus content of the body were increased both by increasing the level of inorganic phosphorus in the diet and by phytase addition. Linear and quadratic relationships between non-phytate phosphorus intake and growth, bone strength, bone mineral content and carcase mineral content were apparent, but

were destroyed by addition of phytase to the diet. A daily intake of 5.5g digestible phosphorus was necessary for maximum growth, whereas 4g/day was sufficient for maximum bone strength. 3.7 g digestible phosphorus/kg diet was recommended for pigs of 10-25kg liveweight. Based on combined criteria it was concluded that adding phytase to a phytate-rich diet at low levels of digestible phosphorus made approximately 70% of the phytate-phosphorus available for bone accretion.

The final trial used pigs growing from 25 to 60 kg liveweight. Levels of non-phytate phosphate were 0.85 (basal diet), 1.25, 1.65, 2.05, 2.45, 2.85, 3.25, 3.65, and 4.05g/kg. 72 individually penned male pigs weighing 25kg were assigned to one of the diets, fed either with or without phytase, at a constant calcium level (8 g/kg).Pigs were slaughtered at 60kg. The left third and fourth metatarsals were removed for breaking force determination and subsequently for mineral analysis.

A lowered daily gain of pigs receiving the basal (0.85g/kg) diet was overcome by the use of phytase and approached that of pigs receiving the 1.65 g/kg diet ($p = 0.015$, sed = 50.1). Linear and quadratic responses of bone strength to intake of non-phytate phosphorus were observed. Daily digestible phosphorus requirement was estimated at 6g for optimum feed conversion efficiency and maximum bone strength, and 9g for maximum growth rate. Addition of phytase resulted in an increased breaking strength of both the third and the fourth metatarsal ($p=0.045$, $p=0.011$, respectively). Based on growth and bone strength data it was calculated that phytase addition to the basal diet enabled 50% of the phytate phosphorus to be utilised. However, the contribution of liberated phytate-phosphorus to that utilised diminished as the dietary phosphorus level increased towards requirement.

It was concluded that addition of *Aspergillus niger* phytase resulted in hydrolysis of phytates, allowing 50-70% of phytate-phosphorus to be utilised, and if used correctly, could substantially reduce phosphorus output from the growing pig sector.

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Chapter 1 Introduction

Concern about the polluting effects of phosphorus has initiated research into optimising the phosphate content of diets fed to pigs. Approximately two thirds of the total phosphorus in pig diets is present in the form of phytates, the mixed salts of myo-inositol hexaphosphoric acid or phytic acid. Phytate bound phosphorus is largely unavailable to pigs, necessitating dietary supplementation with relatively expensive inorganic phosphates such as dicalcium and monocalcium phosphate. In the UK in 1990, 2.4 million tonnes of compound feed was used for pigs, of which inorganic phosphate sources contributed between 8 and 41% of the total phosphorus. While these phosphate supplements are absorbed to variable extents, most of the organic phosphate passes undigested through the animal and is excreted in the faeces. In addition, because the amount of available inorganic phosphorus in the diet is usually above requirement, a proportion of dietary phosphorus is excreted via the urine.

Application of slurry to the land causes leaching of phosphate into the soil, eventually leading to eutrophication of water sources. In Europe, the livestock sector, in particular pig production, has received much of the blame for phosphorus pollution, and has been targeted in an attempt to relieve the phosphorus burden on the land. The North Sea agreement signed by the Paris commission in 1989 committed participating countries to reducing phosphate outputs by 50% between 1985 and 1995. In terms of animal production, particularly in regions of intensive pig production, this can be translated into a gradual reduction in the number of pigs that may be kept in an area.

There are two effective steps that can be taken to alleviate the problem. The first is a closer matching of feed to requirement. The values advocated as phosphorus recommendations differ widely from country to country, as does the methodology used to define these requirements. Furthermore, the data used in computing the requirements, having been obtained from several decades' work, do not reflect accurately the requirements of today's modern fast-growing genotypes. In the short term, a more accurate description of requirements, and a closer matching of the feed to meet these requirements will play some part in reducing the phosphorus output.

The second method is to improve the dietary availability of phytic phosphorus, either by physical processing or with the use of dietary additives such as enzymes. The enzyme phytase (myo-inositol hexaphosphate phosphohydrolase; EC 3.1.3.8.) dephosphorylates phytates, releasing phosphorus for absorption by the animal. Phytase is present intrinsically in most feeds of plant origin, and in animals, the enzyme is produced by microbial flora in the intestine. The contribution of the former to phytate hydrolysis is doubted, as the enzyme is unlikely to survive the acid conditions of the stomach, and the relative quantity of microbial phytase present naturally in the gut precludes it from being significant for phytate hydrolysis in pigs.

Microbial phytase from *Aspergillus niger* has been developed commercially and tested in pigs by several groups of workers. Responses to phytase range from an increase in apparent digestibility of phosphorus and other nutrients, to an improved growth rate, feed conversion efficiency and protein deposition rate. Perhaps the most meaningful aspect of the work to date is that it indicates that performance can be maintained even in the absence of supplemental inorganic phosphate. Increased use of the phosphorus naturally present in feedstuffs leads to the expectation of a reduction in the amount excreted.

Despite these improvements, there remains some scepticism surrounding use of the enzyme, not least because of the variation in results that has been brought about due to a lack of standardisation of conditions, and the use of different sources of enzyme. Furthermore, uncertainty about the actual phosphorus requirement can sometimes constrain satisfactory interpretation of data. At the commencement of this project, research into phytase was in its early stages. Thus this programme was undertaken with two purposes; to further evaluate the effects of phytase on phosphorus availability using selected aspects of pig performance as criteria of response, and as a basis for this, to assess the phosphorus requirement of growing pigs.

Chapter 2 Review of literature

2.1 The phosphorus requirement of pigs

2.1.1 Importance of phosphorus to pigs

The vitalistic nature of phosphorus is manifest in its distribution throughout the body (table 2.1). Phosphorus makes up approximately 1% of the mature body weight of the pig. In the soft tissues, phosphorus occurs as a constituent of cells, membranes and body fluids. Blood plasma contains mostly ionizable phosphorus, which is involved in energy utilization and transfer via adenosine tri-phosphate and adenosine di-phosphate, phospholipid formation and fatty acid transfer, and synthesis of amino acids and protein. Phosphorus is also imputed to be involved in the control of appetite and in the efficiency of feed utilization, by a mechanism as yet unknown.

The imperative requirement for dietary phosphorus is for development and maintenance of the skeleton, in which it is co-precipitated with calcium in the hydroxy-apatite bone complex $[Ca_9^{2+}(H_30^+)_2(PO_4^{3-})_6(OH^-)_2][Ca^{2+}.Mg^{2+}O.3\ CO_3^{2-}.(C_6O_7H_5^{3-})0.3]$. Skeletal tissue undergoes continual accretion and degradation and the resulting growth of the bone is the most important influence on phosphorus balance. Relatively large amounts of phosphorus must be ingested daily in order to satisfy the requirements of the growing pig. Although a severe deficiency is unlikely under commercial conditions, marginal inadequacy can occur with a consequential reduced appetite, bone malformation and lowered fertility.

2.1.2 Absorption of phosphorus

2.1.2.1 Site of absorption

Most phosphorus is absorbed from the small intestine as inorganic phosphate although some phospholipids may be absorbed. Phosphosugars, phosphorylated amino acids and phospho-nucleotides are hydrolysed at the brush border of the enterocyte by alkaline phosphatase, liberating inorganic phosphate which is then absorbed. Intestinal absorption is active and dependent on sodium transport (Berner *et al.*, 1976; Harrison and Harrison, 1961; Taylor, 1974). Absorption occurs most intensively at the proximal half of the small intestine, where solubility is greatest. The peak of absorption after an oral dose of radiophosphorus was 30 minutes after administration, and absorption was almost complete within 3 hours (Guéguen and Rerat, 1967). In porcine tissues, phosphate absorption occurs predominantly in the jejunum and to a lesser extent in the duodenum (Breves and Schroder, 1991). Phosphorus is also secreted into the lumen of the intestine, and reabsorption from the lower segments of the small intestine has been demonstrated (Moore and Tyler, 1955).

The extent of phosphorus absorption from the hindgut is unknown. Despite earlier work (Guéguen *et al.*, 1968) where no absorption of radiolabelled phosphorus from the large intestine could be detected, more recent work (Drochner, 1984; Guéguen *et al.*, 1981) indicated that the absorption of phosphorus from the large intestine should not be neglected. It has been demonstrated in the rat that 8% of total phosphorus absorption occurs in the colon (Cramer, 1961). Results of Partridge (1978a) indicated that in pigs, approximately 12% of total phosphorus absorption occurred beyond the ileum, but this may depend on the dietary phosphorus level. The sites of absorption and reabsorption in the gut are represented by figure 2.1.

2.1.2.2 Mechanism of absorption

Transepithelial phosphate transport consists of an active saturable and a passive non-saturable component. Active transport of phosphorus across the gut wall is represented in figure 2.2. Transport through the luminal cell membrane occurs as an electroneutral cotransport with sodium (Kinne *et al.*, 1977; Quamme, 1985), the low intracellular sodium concentration being maintained by a sodium-potassium pump on the basolateral membrane. Transport of phosphate from the serosa to body fluids occurs independently of sodium, possibly by facilitated diffusion. Evidence of a secondary active sodium-phosphate cotransport system across the brush border membrane of the rat and rabbit small intestine has been found (Quamme, 1985; Berner *et al.*, 1976). The maximum velocity (V_{max}) of this system is increased by a phosphorus deficiency. It is not yet clear whether the carrier preferentially accepts either the monovalent or the divalent form of phosphate.

Measurements of intracellular phosphate transport are complicated by intracellular compartmentalization. In the rat, absorbed phosphate moves through the intestinal cell without entering the cytoplasmic phosphate pool (Kowarski and Schachter, 1969). Thus, absorbed phosphorus could perhaps remain independent of the cell contents by incorporation into vesicles or phosphorylated derivatives.

2.1.3 Renal control of phosphate level

The fate of absorbed phosphorus is influenced largely by the quantity absorbed in relation to requirement. The kidney is the major regulatory mechanism controlling phosphorus level; reabsorption here is saturable, any phosphorus in excess of the maximal transport capacity being excreted into the urine. In addition to phosphorus level, renal regulation depends on intrarenal calcium concentration and acid-base balance. Most plasma phosphorus is in the form of phospholipids and phosphate esters, and thus, unable to filter across the glomerulus into the vascular system. In contrast, inorganic phosphate is almost completely ultrafiltrable at the proximal convoluted tubule, although the cellular mechanisms are unclear. Phosphorus reabsorption in the proximal tubule is highly dependent on the presence of sodium ions in the lumen and may be linked to active sodium ion transport.

It has been suggested (Apfelbaum and Tresmolieres, 1963) that 2 discrete phosphorus pools exist; one dependent closely on phosphorus intake, comprising mainly mineral phosphates with a fast turnover rate, and a second pool independent of intake, containing phosphorus compounds with a slower turnover. Thus excess phosphorus in the diet may be passed directly from the fast pool to urine, without entering the slow pool (figure 2.3). This concept may be extended to include that phosphate with a slow turnover remains independent of cell contents, whereas that with a fast turnover remains freely exchangeable with the blood (Kowarski and Schachter, 1969).

2.1.4 Regulation of absorption and retention

Because the principal regulatory site of blood phosphorus level is the kidney, changes in the dietary supply do not greatly influence absorption percentage. However the subsequent fate of phosphorus is greatly influenced by quantity absorbed, excess over requirement being excreted into the urine, and to a lesser extent, endogenously into the faeces. Dietary factors affecting phosphorus absorption and retention are discussed in *Section 2.2*. An overview of the hormonal influences is given in this section.

2.1.4.1 Parathyroid hormone

Parathyroid hormone (PTH) influences phosphorus balance by depressing reabsorption in the kidney, and may have a minor direct or indirect influence on intestinal absorption. Parathyroid extract increased the transfer of phosphorus from mucosa to serosa by 70%, and uptake by the tissue from the mucosal site by 30% (Borle *et al.*, 1973). However, no effect of parathyroidectomy on absorption or urinary excretion was found in rats (Clark and Rivera-Cordero, 1973).

An increase in intestinal phosphorus absorption in response to dietary phosphorus restriction was not dependent on the parathyroid glands but in parathyroidectomized animals a dose of 0.22 PTH units per kg per hour increased phosphorus absorption from the jejunum from 0.045 to 0.102 mmol/30 minute absorption period (Fox and Care, 1978; Fox *et al.*, 1978). Differences in phosphorus excretion rates in thyroparathyroidectomized rats on low and high phosphorus diets, even when plasma phosphate was controlled by infusion (Bonjour *et al.*, 1973), may indicate that dietary phosphorus activates a mechanism for phosphorus reabsorption other than PTH and plasma phosphorus concentration.

Table 2.1 Phosphorus concentrations in the fresh tissue of the pig

Tissue/Organ	Phosphate concentration
Crude defatted tissue	7.0 g/kg[τ]
Whole blood	20 mg/100ml[τ]
Serum	4.6 mg/100ml[τ]
Skeletal muscle	1.15 g/kg[τ]
Kidney	2.208 g/kg[╫]
Brain	400 mg/kg[╫]
Skin	556 mg/kg[╫]
Liver	381 mg/kg[╫]
Lung	2.112 g/kg[+]

[τ] Georgievski, 1981

[╫] Adapted from Widdowson and Dickerson, 1960

[+] Adapted from Widdowson and Dickerson, 1964

Figure 2.1 Sites of phosphorus absorption

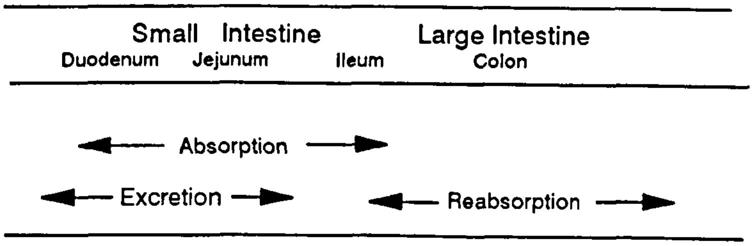

Small Intestine
Duodenum
Jejunum
Ileum
Large Intestine
Colon
Absorption
Excretion
Reabsorption

figure 2.2 Active transport of phosphorus (After Jongbloed, 1987)

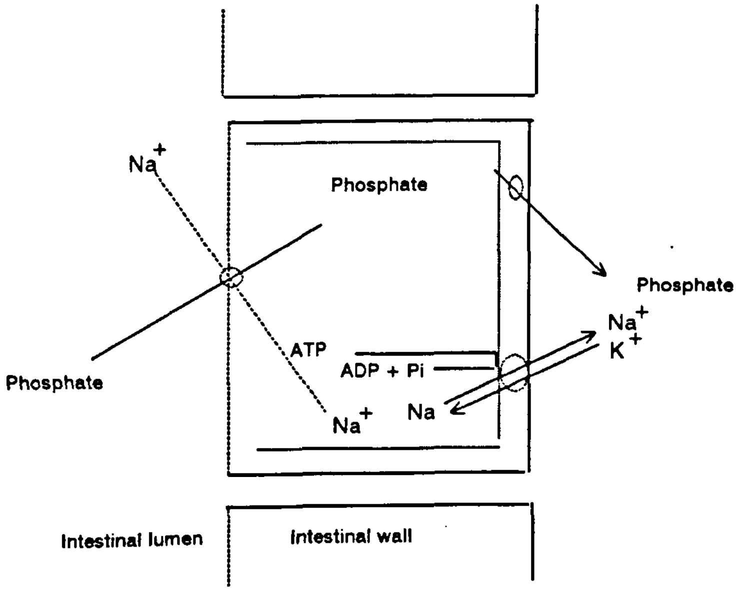
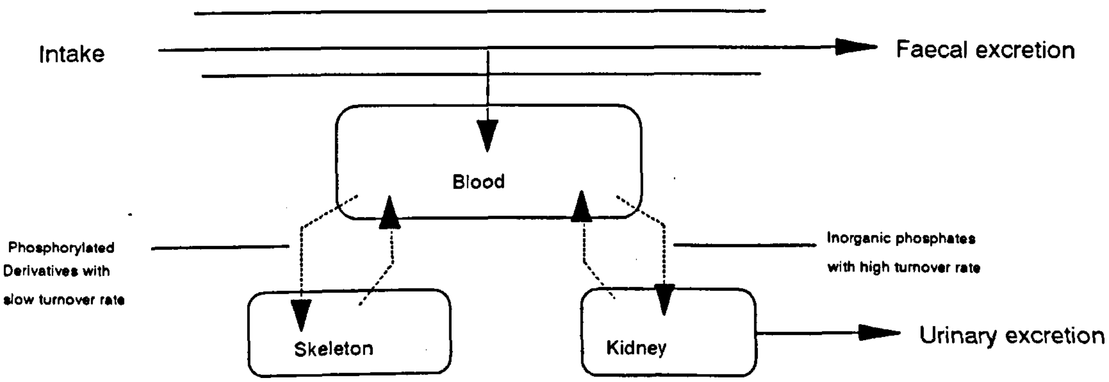

Na+
Phosphate
Phosphate
ATP
ADP + Pi
Na+
Na
Phosphate
Na+
K+
Intestinal lumen
Intestinal wall

Figure 2.3 Model of two discrete phosphate pools - based on concept of Apfelbaum and Tresmolieres (1963) and Kowarski and Schachter (1969)


Intake
Faecal excretion
Blood
Phosphorylated
Derivatives with
slow turnover rate
Inorganic phosphates
with high turnover rate
Skeleton
Kidney
Urinary excretion

In the skeleton, PTH stimulates reabsorption of bone mineral from the matrix, resulting in the release of calcium and phosphorus into the circulation. Calcitonin, which inhibits reabsorption from the ascending loop of Henle and distal convoluted tubule, resulting in a reduced plasma phosphate level, may be more important than PTH for phosphate homeostasis in pigs (Pointillart *et al.*, 1978 a,b). However, there is no evidence that this hormone has a direct effect on intestinal absorption.

2.1.4.2 Thyroxine and growth hormone

Thyroxine may have an indirect influence through its effects on the growth of bone. Injection of thyroxine into rats had no effect on mucosal uptake values but depressed the rate of phosphorus transfer from mucosa to serosa by 32% (Noble and Matty, 1967). Administration of thyroxine (Espinosa *et al.*, 1984) and growth hormone (Hammerman *et al.*, 1984) increased phosphate reabsorption and the activity of the sodium-phosphate cotransport system in the brush border membranes in the kidney.

Growth hormone stimulates endochondral bone formation and increases skeletal mass by accelerating subperiostal bone apposition. The effect of growth hormone on bone is dependent on thyroxine, however, there appears to be no direct effect of thyroxine and growth hormone on phosphorus metabolism.

2.1.4.3 Vitamin D

1,25 dihydroxycholecalciferol (1,25-DHCC), a metabolite of vitamin D, is a potent regulator of calcium and phosphorus movement in the intestine and bone. In rats, stimulation of phosphorus absorption by vitamin D is greatest in the duodenum, decreases at the jejunum and does not occur in the ileum or colon (Lee *et al.*, 1981). Fontaine *et al.* (1985) found that the absorption of phosphorus in vitamin D - depleted pigs was only half of that found in pigs receiving supplementary vitamin D_3 (1000 IU/kg diet). The renal cell responds to excess phosphorus by decreasing fractional phosphate reabsorption. It has been suggested (Bonjour *et al.*, 1977) that the renal cell can adapt more effectively to high dietary phosphorus when vitamin D is present.

Although a direct affect of vitamin D on mineralization has not been shown, in pigs, lack of vitamin D results in a decreased performance, reduced bone ash content and a lowered bone breaking strength. Miller *et al.* (1965 a,b) found that when no vitamin D was given to young pigs, excessive excretion of calcium and phosphorus and thus a lower absorption and retention percentage occurred compared with use of 100 IU/kg diet. When glucose and soya bean protein were used instead of glucose and casein, more than 100 IU per kg diet was required. In the case of growing pigs (10-60kg liveweight) with phosphorus levels of 0.6% or 1.4%, omission of vitamin D from the diet resulted in rickets and a reduced bone ash content occurred. This effect was not seen when the calcium to phosphorus ratio was maintained at 2:1. It has often been suggested that the positive influence of vitamin D on phosphorus absorption may be mediated simply through enhancing calcium absorption, thus reducing the level of calcium ions and decreasing precipitation of insoluble calcium phosphate.

2.1.5 Measurement of phosphorus requirement

2.1.5.1 Empirical versus factorial computation of requirement

Mineral requirements can be assessed using either an empirical or a factorial approach. The former evaluates a range of dietary concentrations using single or multiple criteria. Conventionally, growth rate, feed conversion efficiency, bone composition and strength, and blood components are used as criteria of response, although some of these lack the sensitivity to discriminate between small dietary changes. The factorial method, which computes absolute requirement based on obligatory losses, retention and production requirements, has been proposed by Guéguen and Perez (1981) as scientifically the more satisfactory of the two methods as it can be applied to various systems of production. The dietary requirement is calculated by dividing the sum of these by the phosphorus availability ie, for growth:

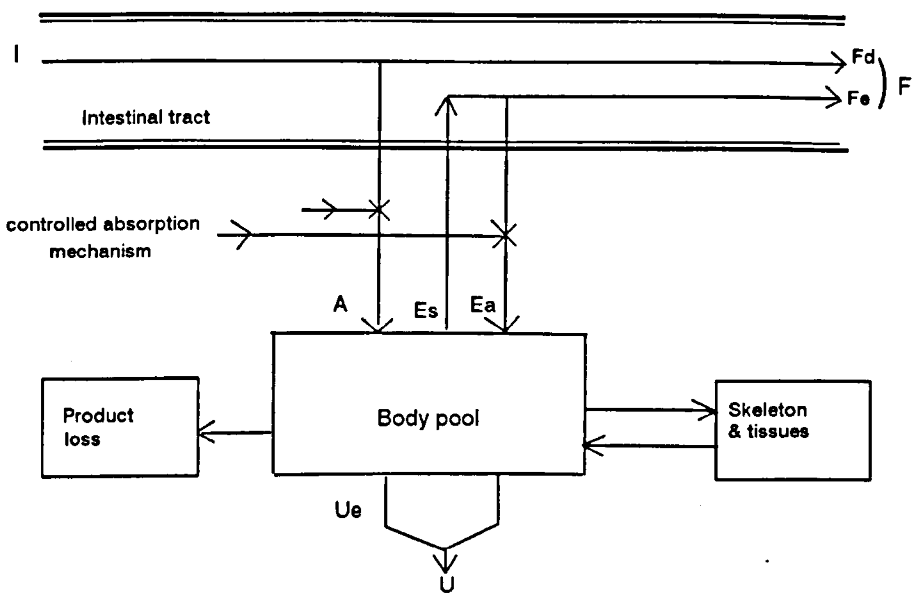
$$\text{Dietary requirement} = \frac{P_{OL} + P_G}{\text{Availability}}$$

where P_{OL} = Obligatory loss of phosphorus
P_G = Phosphorus retained for growth

Such an approach is possible for phosphorus as a result of numerous studies using radio-isotopes which enable the discrimination between undigested and obligatory endogenous losses of phosphorus. The former component of endogenous loss is of dietary origin, excreted to remove excess from the body, while the obligatory loss is defined as the amount excreted at the requirement intake of the mineral. The obligatory loss originates from within the body; from digestive secretions, tissue turnover etc., and increases with age and liveweight of an animal. The amount also depends to some extent on the level of phosphorus supply. Faecal endogenous losses increase up to 15mg/kg liveweight/day when a high phosphorus diet is fed, with an additional 10mg/kg/day urinary loss; thus up to 25mg/kg/day may be required for maintenance on high phosphorus diets. A net maintenance requirement of 5mg/kg liveweight/day has been suggested (Guéguen and Perez, 1981) for pigs receiving a low phosphorus diet.

Figure 2.4 shows schematically the various fractions of mineral during digestion and assimilation. Obligatory endogenous losses together with retained phosphorus constitute the net requirement in the factorial approach. But the assumption in this method that obligatory losses and availability are constant at a given level of intake, and at a particular stage of growth may not be correct. It may be that balance studies can give a more accurate reflection of mineral requirements as they give information concerning digestibility, retention and excretion of phosphorus, and indeed the ARC (1981) concluded that due to uncertainties of factorial estimates, empirically determined values should be given preference.

Figure 2.4 Digestion and assimilation of phosphorus
(After Thompson, 1964)


I
Fd
Fe
F
Intestinal tract
controlled absorption mechanism
A
Es
Ea
Product loss
Body pool
Skeleton & tissues
Ue
U

I = intake of phosphorus
F = total faecal phosphorus excretion
F_d = faecal excretion of unabsorbed phosphorus
F_e = net excretion of phosphorus of body origin into intestinal tract
U = total urinary phosphorus excretion
U_e = excretion of phosphorus into urine at zero net retention
E_s = total endogenous phosphorus secreted into intestinal tract
E_a = endogenous phosphorus reabsorbed from intestinal tract
A = dietary phosphorus absorbed from intestinal tract

2.1.5.2 Criticism of conventional approach

Much controversy exists as to the requirement for growing pigs, due mainly to the varying methodology and criteria of evaluation used in experiments. In many cases, basal diets were not analyzed for phosphorus. Furthermore, intervals between different phosphorus levels in the trial are too large. In addition to changing the level of phosphorus in the diet, calcium:phosphorus ratio also changes, however, ratios less than 1 or greater than 2 lower performance. Performance is often measured at only 2 liveweights, but since, absorption and retention of phosphorus change according to liveweight, several liveweights should be used. Bone parameters are often used as response criteria, but only 75-77% of total body phosphorus is contained within the skeleton, so these may not reflect the phosphorus requirement of the whole body.

The concept of an optimum phosphorus retention needs to be explored. Phosphorus surplus to cellular requirement is stored in the bone and drawn upon during dietary deficiencies, thus the level of phosphorus required for optimum skeletal development is greater than that required for optimal growth. The current practice in the UK of slaughtering pigs at less than 100kg annuls the need for maximum bone mineralization; the definitive question is therefore: "How much bone does a pig require for adequate support if the diet contains adequate phosphorus for other body functions?" On the other hand, although the requirement for growth is probably below that for maximal retention, replacements for breeding stock are often selected from the growing herd, thus potential bone weaknesses at higher liveweights should be avoided. This should be considered when defining the minimum phosphorus level which sustains an acceptable standard of performance.

2.1.5.3 Requirement for maintenance

Maintenance requirement of phosphorus is determined by calculating the endogenous loss in faeces and phosphate losses in the urine. Jongbloed (1987) concluded that the amount of faecal endogenous loss was low and dependent to some extent upon the dietary level. For diets containing adequate levels of phosphorus, a maintenance requirement of 10mg/kg liveweight/day is assumed. However, it has been proposed (Beers and Jongbloed, 1992) that for pigs fed low-phosphorus diets the maintenance requirement drops to 4mg/kg liveweight/day. The absorption coefficient of phosphorus is lower when dietary phosphorus is surplus to requirement. Thus in practise, where animals are fed above their requirement, the maintenance requirement will be slightly higher than that obtained under experimental conditions.

2.1.5.4 Requirement for growth

The amount of phosphorus required for growth can be estimated either from balance data or from carcase analysis. Data from the former approach are presented in figure 2.5 (Woodman and Evans, 1948). The rates of phosphorus gain fell as liveweight increased; this was more pronounced after a liveweight of 30kg was reached. Results from carcase analysis showed that total phosphorus in the body increases with body size (Weniger and Funk, 1953; figure 2.6). The estimated net gain was 4.5g of phosphorus per kg liveweight increase (from 30-150 kg). Jongbloed (1987) claimed that this method was the more accurate for estimating phosphorus requirement for growth, as in most balance experiments phosphorus retention was overestimated.

Net phosphorus requirements estimated by the Agricultural Research Council (1981; table 2.2) are based on liveweight gain, feed conversion efficiency and degree of bone mineralization. Although earlier findings on the net requirement of phosphorus for growth were in agreement, more recent work shows disparities, particularly between "unselected" and fast growing pigs; the latter having a higher net requirement. The mineral composition of gain is more constant in meat-type, less fatty pigs and may increase in lean pigs (Gunther and Rosin, 1970). High daily accretion of protein, usually occurring at high growth rates, can result in an increased phosphorus requirement, hence it may be appropriate to express mineral requirements in relation to body protein and fat gain. In view of the increasing pressure to reduce phosphate output, it is necessary to define more accurately the actual requirement.

Early establishment of a relationship between empty body weight and amount of phosphorus in the body ($P = -64.9 + 9.98\ EBW - 0.05\ EBW^2$; Mudd *et al.*, 1969) suggested a decrease in phosphorus retention per kilogram empty body weight as pigs become heavier. Thus it may be prudent to alter phosphorus concentration of the diet according to liveweight. Jongbloed (1987) determined that the requirement for growth was on average 5.1 g P/kg liveweight gain. Two types of growth were defined; normal (<54% meat in carcase) and very lean (>55% meat in carcase). There was a small non-significant quadratic effect which was taken into account when calculating the requirements, as it was in agreement with previously accepted growth models, and because there was a significant quadratic effect when empty body weight was used as an independent variable. The requirements calculated from this work are given in table 2.3. These indicate higher requirements (approximately 0.25 g/kg body weight gain) for very lean than for normal pig types.

The value of 5.4 g P/kg liveweight gain for pigs from 10 to 20kg is lower than values proposed by Guéguen and Perez (1981; 6.5 g P/kg liveweight gain) and the ARC (1981; 8.5 g P/kg liveweight gain). However, in more recent studies in the Netherlands (IVVO) with piglets a value of 4.9-5.0 g P/kg liveweight gain was found (Dellaert *et al.*, 1990).

2.1.5.5 Dietary phosphorus requirement

The controversy associated with net phosphorus requirement is intensified when dietary recommendations are considered; not least because of the discrepancies arising from estimates about phosphorus availability. Dietary phosphorus recommendations vary widely in different countries, two notable extremes being the ARC (1981) and the National Research Council (1988; table 2.4). ARC (1981) values are based on net requirements calculated to dietary requirements, whereas NRC are based on typical maize-soya diet, where a 50% availability of phosphorus is assumed.

Differences in pig breeds typically used in different countries mean that values obtained from one research institute are not necessarily applicable to pigs in other countries. But in order to recommend any suitable dietary phosphorus level, both the feed intake and the availability of the phosphorus in that feedstuff must be quantified with a degree of accuracy. Generally, estimates of the former are compatible between different research bodies but values ascribed to digestibility can vary markedly. Furthermore, because each individual raw feedstuff has a different phosphorus availability, and because of the diet changes associated with least cost ration formulation, it is not appropriate to assign an availability value to the feedstuff as a whole; the phosphorus availability of each component part must be considered.

Typically, dietary allowances are made up of a net requirement plus a safety margin which allows for performance level differences between animals and between herds, and for lack of sufficient confidence in our estimates of requirements and allowances. The 5-10% safety margin can be lowered only if dietary recommendations can be given with confidence. This relies on valid estimates of phosphate availability.

2.2 Availability of phosphorus

2.2.1 Definition of availability

A precise description of availability has been given as: that proportion of a nutrient source provided in the feed that, at a stated concentration and level of feeding, can be extracted, absorbed and utilized by the animal to meet its net requirements (ARC, 1981). However, a prerequisite for phosphorus to be utilised is that it can be brought into solution in the intestinal tract. Thus in the case of phosphorus, availability is largely a function of the diet, and represents the amount of phosphorus available in the digestive tract. Thus a revised definition of phosphorus availability was adopted by the ARC (1981) as: the proportion of dietary phosphorus that is not combined with compounds that interfere with its digestion, absorption or utilisation by the animal.

This may be represented by the equation:

$$A = I - (F - Fo) - (U - Uo)$$

where A = Availability
I = Intake
F = Total faecal excretion of mineral
Fo = Faecal obligatory loss
U = Total urinary excretion of mineral
Uo = Urinary obligatory loss

The non-phytate portion of dietary phosphorus may be used to give an indication of dietary availability, ie the amount of phosphorus which is available for absorption across the gut wall. This must not be confused with true availability, which is the amount that, once absorbed, is retained in the skeleton or soft tissues. However, it is reasonable to suggest that since phosphorus absorption is not regulated to any great extent at the gut level, most free phosphate will be absorbed across the intestinal membrane. Providing the dietary phosphorus level is not in excess of requirement, and endogenous losses are minimal, dietary availability, or apparent digestibility will give a good indication of the true, or biological availability.

A number of terms have been used to denote the availability of phosphorus in feeds; availability (true and apparent), utilization, digestibility (true and apparent), absorption, and retention. While precise definitions exist, the terms have often been used synonymously without definition. For the purpose of this thesis, apparent digestibility of phosphorus was used as an indication of dietary phosphorus availability. Dietary available phosphorus was defined as: non-phytate phosphorus plus inorganic phosphorus (taking into account the published availability of the inorganic phosphorus). Retention was defined as: the fraction of mineral absorbed into the tissues for growth and production. It was assumed that all soluble inorganic phosphate plus non-phytate phosphorus was absorbed, and that subsequent utilization was dependent on the phosphorus status of the animal, not on the phosphate source.

Figure 2.5 Phosphorus requirement for growth based on balance data (Woodman and Evans, 1948)

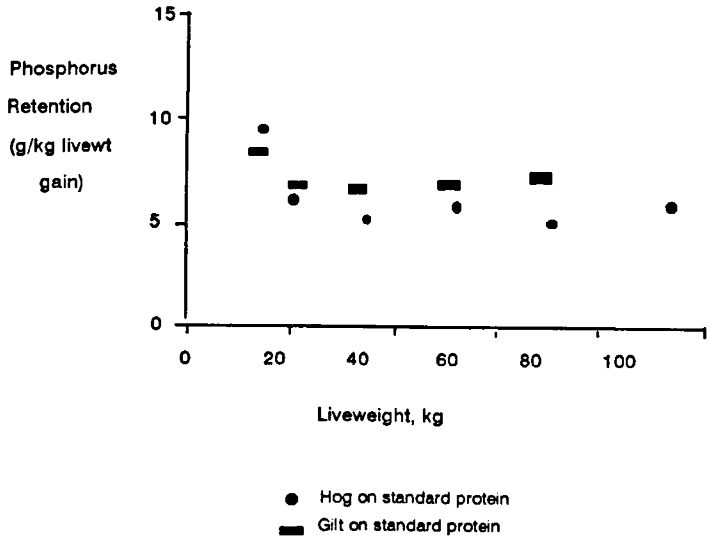


Figure 2.6 Phosphorus requirement for growth based on carcase analysis (Weniger and Funk, 1953)

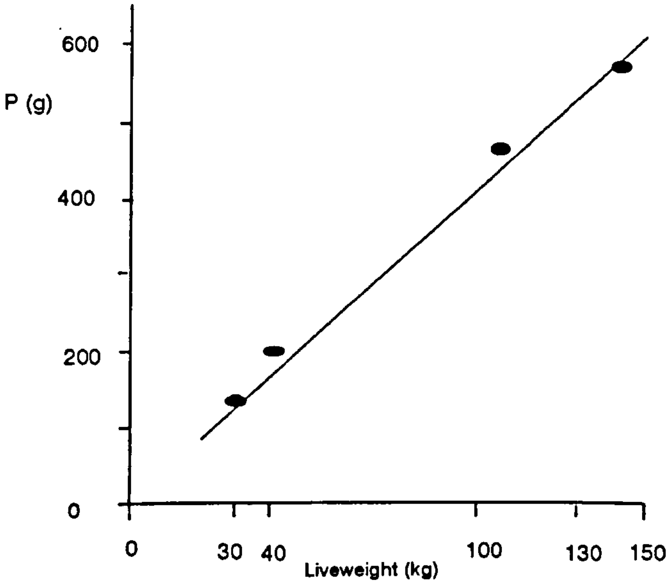


Table 2.2 ARC (1981) estimated phosphorus requirements for growing pigs

Liveweight	*Net requirement (g/day)*	*requirement (g/day)*
5	2.7	3.4
25	4.6	6.1
45	5.2	7.4
90	5.8	8.9

Table 2.3 Calculated phosphorus requirements for growth (g/kg liveweight gain; Jongbloed, 1987)

Liveweight	10	30	50	60	70	90
Normal	5.13	5.08	5.05	5.03	4.98	4.93
V. lean	5.45	5.38	5.35	5.35	5.33	5.28

Table 2.4 Dietary phosphorus recommendations of ARC (1981) and NRC (1988)

Liveweight	10-25kg	25-50kg	50-100kg
		total phosphorus in diet g/kg	
ARC (1981)	7.8	6.3	5.8
NRC (1988)	5.3	4.9	4.2

2.2.2 Measurement of phosphorus availability

The total content of phosphorus in any dietary source gives little indication of its usefulness to the animal unless it is accompanied by a coefficient denoting its availability to the animal. The methods most commonly associated with assessment of the availability of phosphorus in foodstuffs are the slope ratio and the balance methods. A slope ratio assay involves comparing the slope of the response of growing pigs to graded levels of the test phosphorus with the slope of a response to a standard phosphorus (figure 2.7). Monosodium phosphate ($NaH_2Po_4.H_20$) is used as the reference and the availability of its phosphorus is assumed to be 100%. The criterion of response is usually bone breaking strength, bone ash percentage, or phosphorus retention in the empty body. Blood phosphorus and alkaline phosphatase activity are also used, although these are not very sensitive and do not show a linear response to the amount of phosphorus absorbed. In addition, they may be influenced by diurnal variation and stress during sampling. In young, growing animals, daily liveweight gain and feed conversion efficiency can also be used, although these indicators are assumed to be less sensitive than bone parameters or phosphorus in the empty body.

Inconsistencies in values have been reported (Ketaren *et al.*, 1993), depending on whether bone bending moment or phosphorus retention in the empty body is used. Further inaccuracies can occur because monosodium phosphate is assumed to be 100% utilizable, but only approximately 90% is digested, and a percentage of this is utilized. Work by Dellaert *et al.* (1990) suggests that availability figures obtained by the slope ratio technique (using monosodium phosphate as a reference) should be multiplied by 0.9 to obtain true digestibility coefficients.

The balance technique uses phosphorus digestibility as an estimate of availability; this has been found to have a higher correlation with retention than blood or bone related parameters (Dellaert *et al.*, 1990) and can therefore be used as a reliable estimate for the nutritive value of phosphorus in feedstuffs. Two assumptions are made; firstly, that dietary phosphorus concentration does not affect digestibility and secondly, that all digestible phosphorus can subsequently be utilized. This technique does not consider endogenous losses which are used in tissue turnover and are therefore excreted and measured as undigested. Thus the balance method measures apparent digestibility as opposed to true digestibility.

True digestibility can also be measured using radiolabelling techniques; this gives a precise indication of mineral availability. Methodology for determination of true digestibility has been reviewed by Whittemore (1970) and can be summarised as:

- *Specific nutrient inanition* which measures the output of mineral in the faeces of animals fed on diets free of the element
- *Regression* of the mineral balance against mineral intake of an animal. Animals are fed different levels of the same mineral source, and the constant of the regression indicates the endogenous faecal excretion.
- *Isotope dilution* which involves the injection of a tracer dose of the radio isotope into the vein or muscle, causing the element to become uniformly labelled throughout the body.
- *Comparative balance* involving a pair of animals, one dosed orally and one intravenously, to determine the endogenous faecal loss.
- *The urine ratio method* based on a modification of the above comparative balance method, using mineral loss in the urine to calculate the proportion of the oral dose which, after being absorbed, is excreted in the urine.
- *The carcase ratio method*, again, a modification of the comparative balance, using isotope recovery from the whole carcase as an estimate of availability.

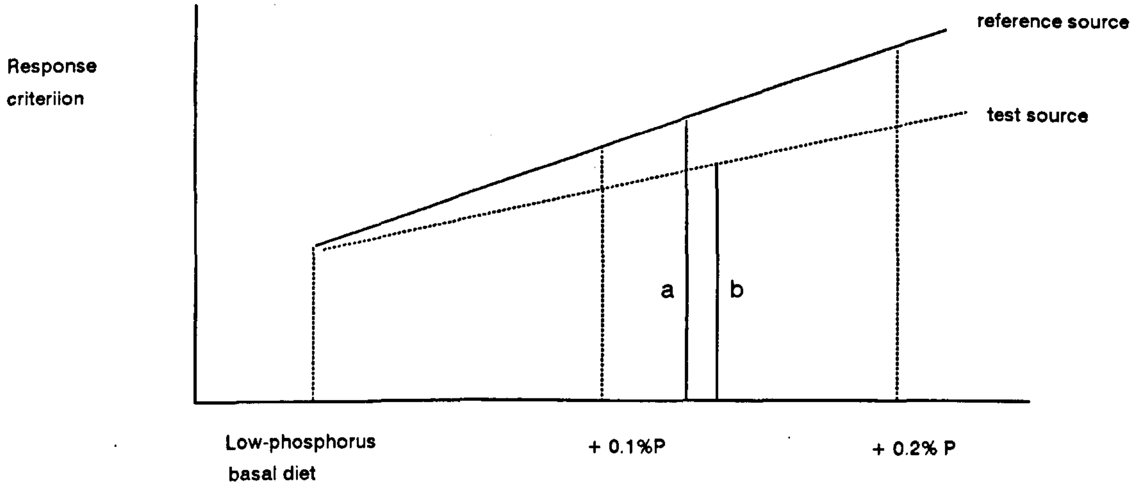
The latter two techniques avoid the need for faecal endogenous data by determining directly the movement of phosphorus across the gut. Measurements of true digestibility involve use of the heavy isotope ^{32}P, which is expensive and impractical to use, and precludes the use of many replicates. In view of these difficulties, it may be more practical to use balance data and to correct for endogenous losses to obtain true digestibility, or alternatively to use apparent digestibility of a low phosphorus diet for which it is known that endogenous losses will be obligatory only.

For the feed mineral industry, studies on biological availability are expensive and time consuming, and thus chemical tests are used. No chemical test exists which gives an unequivocal correlation to biological availability, instead, comparative evaluations are made on the basis of reactivity and solubility of different feed phosphates. The tests that are used are:

- determination of total phosphorus content
- determination of phosphorus soluble in 2% citric acid
- determination of phosphorus soluble in neutral ammonium citrate
- determination of phosphorus soluble in alkaline ammonium citrate at ambient temperature (Petermann method)
- determination of phosphorus soluble in water (only for products containing water soluble phosphorus)

For water-insoluble phosphates, it has been proposed (Guéguen, 1977) that solubility in citric acid is the most reliable estimate of bioavailability of water-insoluble feed phosphates, even if it does not discriminate well between highly available phosphates.

Figure 2.7 Slope ratio bioassay for measurement of phosphorus availability



Relative biological value of test source = b/a x 100

2.2.3 Effects of dietary constituents on phosphorus availability

2.2.3.1 Mechanism of interaction

As was previously indicated, availability of phosphorus is largely a function of the source of the mineral. In the digesta, minerals can exist in three forms: as metallic ions in solution, as constituents of metallo-organic complexes in solution, or as constituents of insoluble compounds. Ions are readily absorbed, whereas metallo-organic complexes have variable digestibility. Insoluble compounds are not absorbed at all. The relative availability of phosphate from vegetable, animal and mineral origin is discussed fully in *Section 2.3.*

The composition of the diet also plays a role in determining phosphorus availability. Phosphate in its soluble or partly solubilised form interacts with many of the other nutrients in the digestive tract. These interactions can be either antagonistic, as in the case of calcium and phosphorus, which inhibit the absorption of each other, or synergistic, where the elements mutually enhance their absorption in the gut and jointly fulfil some metabolic function at the tissue or cell. The effects can be direct or indirect. For phosphorus, the following associations are potentially significant:

- interaction through intermediary phosphorylation processes in the intestinal wall and the activity of digestive enzymes (ie the effect of phosphorus on liberation from the feed and the absorption of other elements)

- direct interaction with calcium at the tissue level to form hydroxyapatite

- activation of enzyme systems and intensification of synthetic processes requiring the presence of other minerals.

2.2.3.2 Calcium to phosphorus ratio

In pigs, interactions between phosphate and calcium are perhaps of most importance. Although transport of phosphorus is not coupled to calcium, it is unclear whether the presence of calcium is necessary for phosphorus uptake. For skeletal growth, calcium and phosphorus should be present in body fluids in the ratio of 2.2(Ca):1(P). An excess of either causes formation of insoluble calcium phosphate. However, while small increases in the ratio may lower the proportion of phosphorus absorbed with respect to that ingested, final retention is not significantly altered (Whiting and Bezau, 1958; Guéguen and Rerat, 1965). ARC recommended ratios are shown in table 2.5

The emphasis which has been placed on calcium:phosphorus ratio tends to lead to the assumption that use of the correct ratio will ensure that the animal's requirement is met. However, optimal Ca:P ratio is valid only when dietary levels are supplied in the correct amounts, and varies with the levels of calcium and phosphorus in the diet. It has been suggested (Whittemore, 1970) that the influence of calcium:phosphorus ratio should be considered both in relation to the intestinal absorption, and absorption into the body pool. The minerals are mutually antagonistic at the gut level but must be present in the ratio 2:1 in the blood to form the hydroxy-apatite complex.

2.2.3.3 Magnesium

Based on the observation that a magnesium deficiency reduced the concentration of serum phosphorus and increased urinary phosphorus, it was proposed (Lifshitz *et al.*, 1967) that magnesium indirectly affects the phosphorus transport system. When both dietary calcium and phosphorus were low, magnesium did not effect phosphorus absorption, but as the phosphorus level increased, absorption was stimulated by magnesium (Clark, 1968). Stimulation of calcium absorption by magnesium would decrease the amount of calcium available for precipitation in the intestine, which may cause an increased phosphorus absorption. Very low magnesium levels are unusual in practical pig feeding; an excess of magnesium is more likely. In most experiments, an increased magnesium level decreased urinary phosphorus excretion (Clark and

Bélanger, 1967; Clark, 1968; Ballavia and Wallach, 1973; Pointillart and Guéguen, 1973; Rogel and Chenoweth, 1976). Formation of a magnesium-phosphorus complex in the gastro-intestinal tract has been postulated (O'Dell, 1960) but it may be that the antagonism between phosphorus and magnesium occurs at the renal level.

2.2.3.4 Protein

Protein can influence the absorption and retention of phosphorus by altering growth rate; a higher retention of protein results in an increased phosphorus requirement because of its relatively high concentration in the fat-free soft tissues. Increased absorption and retention of phosphorus when protein level is raised has been demonstrated (Hendricks *et al.*, 1970; Mulller and Kirchgessner, 1974). Thus far, there have been few experiments to study the effects of individual amino acids on phosphorus absorption. L-phenylalanine, an inhibitor of alkaline phosphatase, inhibited intestinal radiophosphorus absorption in chicks (Wasserman and Taylor, 1973) as did lysine (Chow *et al.*, 1972).

2.2.3.5 Energy level

Assessment of the effects of energy level on mineral uptake is confounded by the inability to maintain a constant mineral intake and alter the energy level, whilst feeding the same diet. When the concentration of dietary phosphorus was adjusted to achieve a constant daily mineral intake, it was found (Moinizadeh, 1975) that retention percentage increased with energy intake, up to a maximum of 3.5 x maintenance (table 2.6). In contrast, no differences in phosphorus absorption or retention were observed between pigs fed at a high or low level of the same diet (Sauer *et al.*, 1982). If an increased feeding level is to result in fat synthesis, a concomitant increase in mineral intake may not be necessary. However, muscle and bone development require phosphorus, thus, for growing pigs it may be necessary to increase the mineral level in proportion to energy.

Table 2.5 ARC (1981) recommended calcium to phosphorus ratios

Liveweight	calcium to phosphorus ratio
up to 20 kg	1.7:1
20 - 55 kg	2.0:1
55 - 90 kg	2.4:1

Table 2.6 Effect of energy supply on phosphorus retention (Moinizadeh, 1975)

Energy Supply[a]	Retention (%)
2.0	8.6
2.5	14.6
3.0	16.0
3.5	18.2
4.0	17.6

[a] = multiples of maintenance requirement ($M = 0.65W^{.569}$ MJ Nef)

2.2.3.6 Fat

Saponification occurring between calcium and long chain saturated fatty acids may facilitate phosphorus absorption; furthermore, enhancement of fat-soluble vitamin absorption may have a positive effect on phosphorus uptake. An increased phosphorus absorption was found when dietary fat was raised from 3% to 15% (Jφrgensen and Fernandez, 1984) however the concomitant increase in energy level may have influenced absorption. No alteration in phosphorus absorption was found with addition of fat by Gundel and Kemenez (1980) or by Holler and Hill (1968, 1969).

2.2.3.7 Carbohydrates

Glucose is essential as an energy source for phosphorus absorption, however pig feeds are generally sufficient in starch and sugar so that their effects on phosphate uptake are not of concern. Structural carbohydrates interfere with phosphorus absorption by acting as cation-exchange resins to bind minerals, effectively reducing mucosal concentration and increasing intestinal phosphorus secretion. Properties of cellulose other than its cation-binding capacity may be involved in its ability to reduce mineral absorption, for example, the increased rate of passage associated with fibre may reduce the opportunity for mineral absorption.

The binding capacity for phosphorus decreases from lignin -> hemicelluloses -> cellulose (Bagheri *et al.*, 1982). Phosphorus absorption from the terminal ileum was found to be negative and further depressed by addition of cellulose and lignin (Drochner, 1984) which suggests that intestinal mineral secretion is enhanced by fibre. However it has been proposed (Partridge, 1978b) that the reduction in apparent phosphorus digestibility occurs in the large intestine.

2.3 Availability of phosphorus in pig feeds

2.3.1 Availability in feeds of plant origin

While early estimates of availability were 30-35% in phosphates of plant origin, it is now realised that a much wider spread exists. In fact, the availability of phosphorus in feedstuffs of plant origin varies from around 10 to around 50%, and it is generally assumed that at least two thirds of organic phosphorus in the diet is unavailable to the pig. Table 2.7 gives recent estimates of phosphorus digestibility from different feedstuffs of plant origin.

Most phosphorus in most plant sources is present in the form of phytates which are the mixed salts of myo-inositol hexaphosphoric acid, or phytic acid (1,2,3,4,5,6-dihydrogen phosphate myo-inositol; IUPAC-IUB, 1968). It has been suggested (Nelson, 1980) that a close approximation to the available phosphorus content of the diet is the non-phytate portion. Frequently the non-phytate phosphorus is termed 'available' and represents the amount of phosphorus which the animal can digest.

2.3.1.1 Phytate content of feedstuffs

Phytic acid content of cereals, oilseeds and their protein products is shown in table 2.8 . Phytate content of cereals ranges from 0.50 to 0.89% of the dry weight, and from 0.4 to 5.2% in legumes and oilseeds, containing up to 88% of the total phosphorus present. In general there is a high degree of correlation between total phosphorus content and phytic acid level. Protein products derived from phytate-containing seeds are also high in phytate.

In most seed types, the phytic acid is associated with specific components; O'Dell *et al.* (1972) reported a level of 0.32% phytate in the whole kernel of wheat; approximately 87% associated with the aleurone layer, 13% in the germ and 2% in the endosperm. Lolas *et al.* (1976) found a range of 4.59 to 5.52% phytic acid in wheatbran as compared with 0.62 to 1.35% in the whole kernels. In corn, almost 90% of phytic acid is concentrated in the germ portion. In oilseeds, which contain little or no endosperm, the phytates are distributed throughout the kernel, located within aleurone grains. A range of 0.54-1.58% phytic acid has been reported in beans (*Phaseolus vulgaris*; Lolas and Markakis,1975) and there exists a high degree of correlation between total phosphorus content and phytic acid level.

2.3.1.2 Structure of phytic acid

9 stereoisomeric forms of inositol exist; *myo*-inositol, *epi*-inositol, *allo*-inositol, *cis*-inositol, *scyllo*-inositol, *neo*-inositol, *muco*-inositol, *D* chiro-inositol and *L* chiro-inositol. *Myo*-inositol is the most abundant naturally occurring inositol; the structure proposed by Anderson (1912; cited in Erdman, 1979) is shown in figure 2.8. Phytate present in various feeds has different characteristics which may influence its potential hydrolysis and subsequent release of phosphorus. Phytate in mature seeds is myo-inositol hexaphosphate, but the native state is not the same for all seeds.

Differences in the solubility of phytate from different sources (De Boland *et al.*, 1975), suggests differences in the degree of hydrolysis and subsequent utilisation of phytate-phosphorus by animals. These differences in solubility of phytate from different sources affect the extent of enzyme degradation and subsequent utilization by the animal. Availability of corn phytate-phosphorus is low (12%) whereas that from wheat is approximately 48% available (as estimated from bone strength measurements; Cromwell, 1980).

2.3.1.3 Mechanism of phytate interaction with other nutrients

Once ingested, dietary phytic acid is soluble and rapidly reacts with cations, forming insoluble complexes containing calcium and a trace element. Variability of phyate stereochemistry increases reactivity in the gastro-intestinal tract. Weingartner and Erdman (1978) proposed a partially dissociated Anderson-based structure occurring at neutral pH when phosphorus groups have either 1 or 2 negatively charged oxygen atoms (figure 2.9), enabling strong chelation of cations between two phosphate groups, or weaker binding within a phosphate group (figure 2.10).

The solubility of phytate-mineral salts is pH dependant, increasing as the pH decreases from 6 to 3.5. At pH 7.4 phytic acid complexes with metals in the following decreasing order of stability: $Cu^{2+} > Zn^{2+} > Co^{2+} > Mn^{2+} > Fe^{2+} > Ca^{2+}$ (Meddaiah *et al.*, 1964). However at the duodenum, where maximum absorption of divalent metal ions takes place, the pH is more acidic (approximately 6.0) and interaction between these ions and calcium-phytate occurs, resulting in precipitation of mixed calcium phytate complexes; the extent of which depends on the presence of high concentrations of each constituent in the solution of the intestine. A synergistic effect between metal ions has been demonstrated *in vitro* (Oberleas, 1973). When two or more cations are present simultaneously the quantity of metallic phytate precipitated is increased. These metal-bound phytates are not readily hydrolysed in the pH range of the digestive tract.

Reduced availability of essential minerals by phytate and phytate-protein complexes depends on concentration of phytic acid and minerals in the feed, hydrolysis of phytate in the intestine, ability of endogenous carriers in the intestine to absorb minerals bound to phytate, presence of inhibitors of hydrolysis and effects of processing. Only soluble phytate can be hydrolysed (Hill and Tyler, 1954). After hydrolysis, the lower inositol phosphates are less able to precipitate with calcium at neutral pH in vitro (Thomas and Tilden, 1972) so hydrolysis would lead to increased solubility of metals in the intestine.

Table 2.7 Apparent phosphorus digestibility of feedstuffs of vegetable origin

Feedstuff	P (g/kgDM)	Mean	Range
Barley	4.4 ± 0.3	39	34-44
Maize	3.2 ± 0.4	17	12-26
Wheat	4.1 ± 0.4	47	45-51
Wheat middlings	12.0 ± 0.8	28	18-35
Maize gluten feed	9.8 ± 1.6	20	12-32
Maize meal solv. extr.	7.4 ± 0.8	20	11-31
Peas	4.8 ± 0.9	45	42-51
Beans (Phaseolus sp.)	5.2 ± 0.4	38	29-48
Soyabean meal solv. extr. (XF> 7%)	6.6 ± 0.5	37	36-38
Soya bean meal solv. extr. (XF<3.5%)	7.3 ± 0.6	38	33-41

Source: (Jongbloed *et al.*, 1991)

Table 2.8 Phytic acid phosphorus content of feedstuffs

Feedstuff	Phytic acid P (% of DM)	Phytic P % of total P
Barley	0.28	64
Maize	0.21	66
Wheat	0.29	71
Wheat middlings	0.96	80
Maize gluten feed	0.63	64
Maize meal solv. extr.	0.54	73
Peas	0.24	50
Beans (phaseolus sp.)	0.17	33
Soyabean meal solv. extr (XF>7%	0.40	61
Soyabean meal solv. extr.(XF<3%)	0.42	58

Source: (Jongbloed *et al.*, 1991)

Figure 2.8 Anderson structure of phytic acid

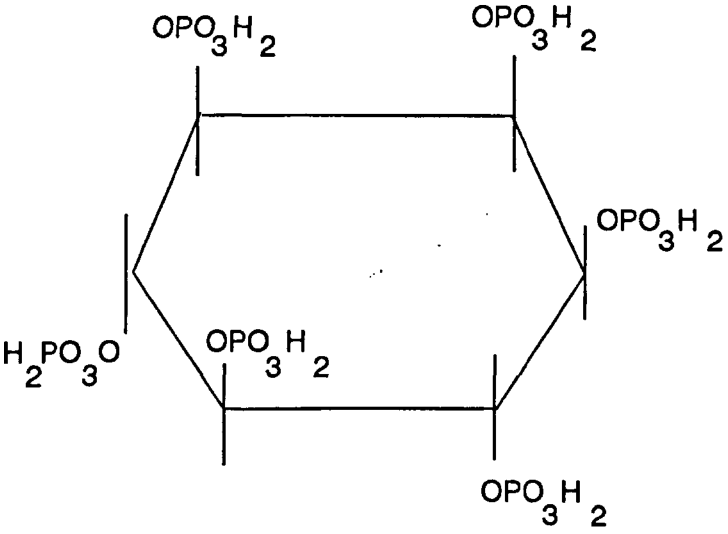

OPO_3H_2
OPO_3H_2
OPO_3H_2
H_2PO_3O
OPO_3H_2
OPO_3H_2

Figure 2.9 Partially dissociated Anderson-based structure of phytate allowing chelation of cations between phosphate groups (Weingartner and Erdman, 1978)

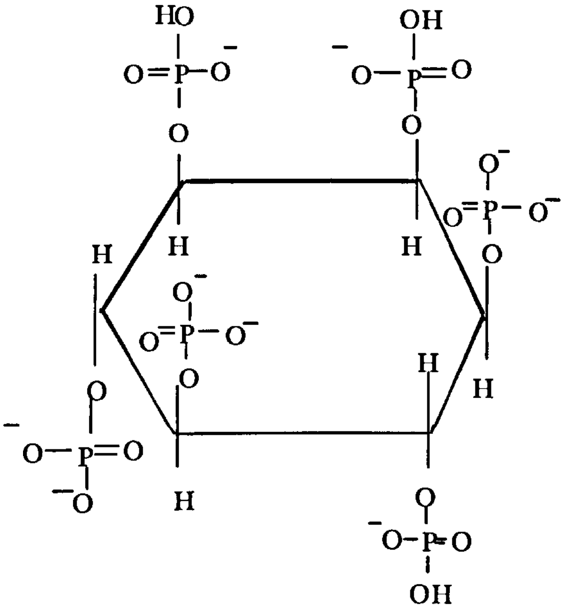
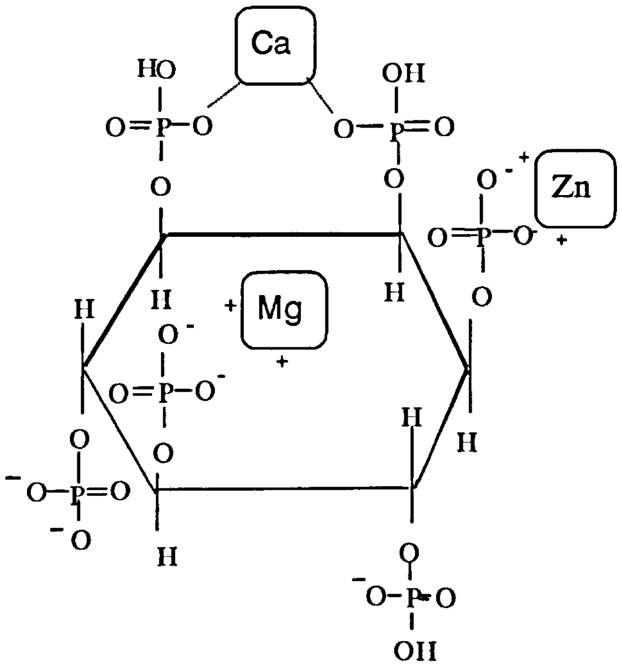


Figure 2.10 Binding of cations within a phosphate group (Weingartner and Erdman, 1978)



2.3.1.4 Calcium-phytate interactions

Calcium is the most abundant divalent cation in most pig diets, and calcium-phyate is therefore predominant. Solubility of calcium phytate is low, dissolving at pH4 at a molar ratio of 6:1 (calcium: phytate; Wise and Gilburt, 1981). The fate of soluble phytate in the intestine will depend on whether it is hydrolysed to yield inorganic phosphorus or becomes insoluble thus preventing hydrolysis. The concentration of calcium determines the solubility and thus the fate of phytate. When there is a low calcium to phytate ratio in the diet, the majority of the products of phyate hydrolysis appear to be metabolised. Nahepetian and Young (1980) found that a large percentage of ^{14}C-labelled calcium phytate was exhaled as $^{14}CO_2$ by rats. Furthermore, when mice were fed a diet containing a low ratio of calcium to phytate, a high percentage of the phosphorus present in the phytate was absorbed; that which was not utilized was subsequently excreted in the urine (Yoshida *et al.*, 1987).

2.3.1.5 Magnesium-phytate interactions

Early reports (Roberts and Yudkin, 1960) suggested that magnesium deficiency symptoms could be aggravated by addition of sodium phytate to casein-based diets. A mixture of calcium and magnesium with phytate leads to precipitation of the mixed salt phytin. It has been suggested (Wise, 1983) that the ratio (calcium + magnesium): phytate determines the fate of phytate in the intestine. At low calcium concentrations, phytate is not precipitated and can therefore be hydrolysed. As calcium increases, calcium phytate precipitates and the phytate passes to the caecum where it may be hydrolysed by hind-gut bacteria.

2.3.1.6 Interactions between phytic acid and other minerals

Numerous studies have implicated phytic acid as a causative factor in poor zinc absorption from plant foods. Likuski and Forbes (1965) established an inverse relationship between phytic acid content and zinc bioavailability of animal diets.

At pH values of the gut, zinc forms the most stable (insoluble) metal complex with phytic acid. It has been suggested (Rackis and Anderson, 1977) that differences in zinc bioavailability are most likely to be due to the phytate-protein-mineral interactions formed during processing, rather than specific phytate contents. Trace minerals such as copper, manganese, molybdenum and cobalt may also be affected by phytates, leading to imbalances due to preferential binding. Strong binding of zinc to dietary phytic acid to form insoluble complexes would favour copper absorption and therefore change the zinc:copper ratio (Klevay, 1977).

2.3.1.7 Phytate-protein interactions

Most phytate in stomach acid is either soluble or complexed with protein; on its arrival in the small intestine it may co-precipitate as the metal-phytate complex due to neutralization of the acid. Pepsin digestion of casein and bovine serum albumin were significantly ($p < 0.05$) decreased in the presence of phytate; this reduction was linearly dependent on phytate level (Knuckles *et al.*, 1985; figures 2.11 and 2.12). Similar effects of phytate on protein digestibility have been reported by Barre (1956). Decreased digestion of proteins in the presence of phytate hydrolysates suggest that other inositol phosphate esters may affect protein digestion.

Phytic acid forms complexes with proteins in both acidic and alkaline conditions. At low pH the negative charge on the phytate interacts with the positive amino groups of protein, whereas at high pH multivalent cations, for example Ca^{2+}, mediate complexing between protein and phytate. When soybean protein (high phytate) was digested, the proportion of insoluble nitrogen in the duodenum was 50% as compared with 28% from a casein-gluten source (Zebrowska, 1978). It is possible that the protein-calcium-phytate complexes are favoured in the presence of low calcium concentrations and that these are more soluble than calcium phytate.

Figure 2.13 shows the structure of a phytic acid-protein complex at alkaline pH proposed by de Rham and Jost (1979). Fontaine *et al.* (1946) suggested that up to approximately pH 3.5, most of the phytate is bound to proteins, however, as proteins pass through their iso-electric points, the complexes dissociate. Above pH7, there may be re-association of phytates with protein. The strength of binding between phytate and proteins varies with protein source (O'Dell and de Boland, 1976; Erdman, 1979), probably due to differences in protein configuration.

The interaction between protein and phytic acid is thought to be ionic (de Rham and Jost, 1979) and causes reduced solubility of proteins; thus inhibiting proteolytic degradation. Calcium ions interact with protein and phyate to further decrease solubility (Saio *et al.*, 1967). Complexing between soya protein and phytate was disrupted when the calcium ion concentration was increased at neutral pH, and calcium phytate was precipitated (de Rham and Jost, 1979).

2.3.1.8 Interactions between carbohydrates and phytate

There may be an effect of phytate on starch digestion in the gut. At pH 6.9 and 4.2 *in vitro*, phytate reduced starch digestion by salivary amylase to 78.3% of the control (Knuckles and Betschart, 1987). However, the effect of phytate on starch digestion differs with enzyme source. The effect appears to be due to non-competitive interactions of the ester with the enzyme; possibly by phytate blocking the active sites. Other more highly phosphorylated inositol phosphate esters from hydrolysed phytic acid may also affect the enzyme digestion of starch. Cereal fibre is closely associated with phytic acid, and some of the effects of fibre are often confounded with phytic acid. There may be an interactive effect between soluble fibre and phytate to further decrease phosphorus availability.

2.3.2 Availability of phosphorus from animal sources

In a series of balance trials (Jongbloed and Kemme, 1990a) apparent digestibility coefficients were determined for products of animal origin. The results, together with results of Dellaert *et al.* (1990) using the slope-ratio technique, are given in table

2.9). Although availability of phosphorus in feedstuffs of animal origin is higher than that of vegetable origin (68-91%), total phosphorus content can vary by as much as 30%. Thus the amount of phosphorus obtained from these sources is often overestimated.

2.3.3 Availability of inorganic phosphates

The contribution of feed phosphates of mineral origin must not be ignored. Commercial phosphates are manufactured by dissolving apatite with an acid, followed by purification and extraction of the product. In recent years there has been a sharp increase in phosphate rock prices, with an accompanying increase of feed phosphate price. Consequently, more attention has been drawn to biological availability. Several types of phosphates are produced commercially, with different availabilities, reflecting differences in their chemical configuration (for example, particle size and crystallinity). Concentration of other elements and pH also influence availability of the various phosphates. It has been shown that for feed phosphates, digestibility of phosphorus has a higher correlation with phosphorus retained than blood or bone-related parameters (Dellaert *et al.*, 1990). An extensive review of phosphorus availability from various mineral sources was undertaken by Jongbloed (1987), the results of which are summarised in table 2.10.

2.3.4 Implications of low phytate-phosphorus availability

Although supplementation of highly available phosphates into the diet satisfies the requirement of the pig, it does not address the matter of pollution. Pig slurry contains 1-2% phosphorus (DM basis) of which up to 60% can be of organic origin (Gerritse and Zugec, 1977). In Britain, the pig sector contributes approximately 47,000 tonnes of phosphate annually to the already critical burden on the environment. While it is possible to supplement the diet with a highly available phosphate source at the expense of those with a lower availability, it may be that limitations imposed on the that limitations imposed on the inclusion of ingredients in pig diets will cause these ingredients to be used elsewhere. Thus the a lowered phosphorus excretion by pigs may be outweighed by an increased excretion from other species.

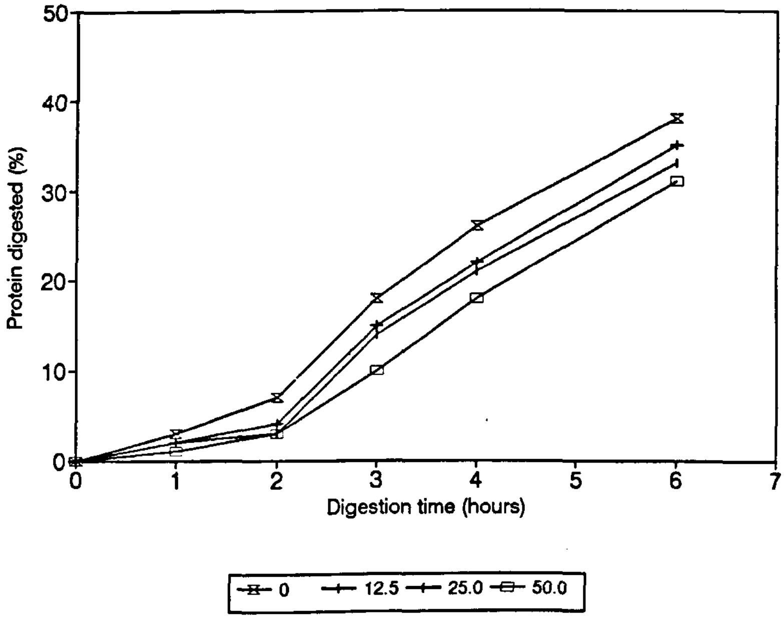
In order for the phosphate output to be lowered to an acceptable level, three steps must be taken. Firstly, the requirement of growing pigs must be more critically defined. Experimental error must be accounted for and a consensus reached between the different research bodies as to the actual requirements, rather than averaging values from a diverse range of experimental conditions. Secondly, close attention should be paid to the availability of phosphorus in feedstuffs, and diets formulated taking these into consideration. And thirdly, ways of improving the availability of phytate-phosphorus must be explored.

2.3.5 Hydrolysis of phytates

Although phytates are stable compounds, some degradation may occur during processing and/or subsequent digestion. Breakdown of plant cell membranes, and release of phytates from the globoid bodies exposes them to hydrolysis. Early experiments (Averill and King, 1926) showed that dry or moist cooking decreased phytate content of ingredients. More recently, it was reported that pelleting increased the utilisation of phytate phosphorus by 25% (Summers *et al.*, 1967). During processing, some phytate is hydrolysed to other myo-inositol phosphate esters, varying in degree of phosphorylation depending on pH and temperature.

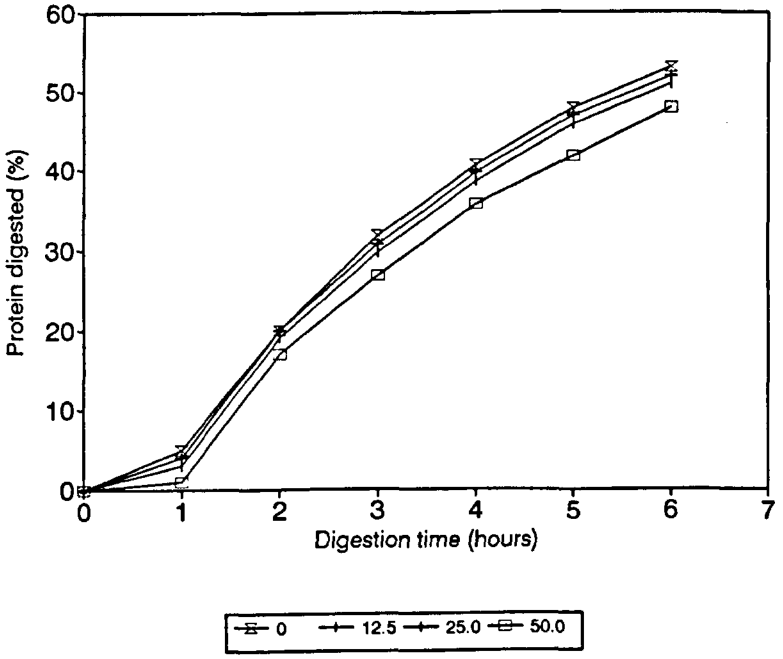
If phytate in feed becomes soluble in the intestine, its subsequent fate will depend upon whether it is hydrolysed to yield inorganic phosphate or becomes insoluble, thus preventing hydrolysis. Either way, the amount of phosphorus released is small compared with the animal's need. Figure 2.14 shows the rate of loss of phytate during autoclaving inositol hexaphosphate and various natural products at 115°C (de Boland *et al.*, 1975). Removal by this method is impractical; 30 minutes' autoclaving reduced phytate content of cereals and oilseeds by less than 10%.

Figure 2.11 Reduced digestion of casein in the presence of phytate (Knuckles *et al.*, 1985)



Phytate levels 0,12.5, 25 and 50 g per 0.5mg casein

Figure 2.12 Reduced digestion of bovine serum albumin in the presence of phytate (Knuckles *et al.*, 1985)



Phytate levels 0,12.5, 25 and 50 g per 0.465mg BSA

Figure 2.13 Structure of phytic acid-protein complex at alkaline pH (de Rham and Jost, 1979)

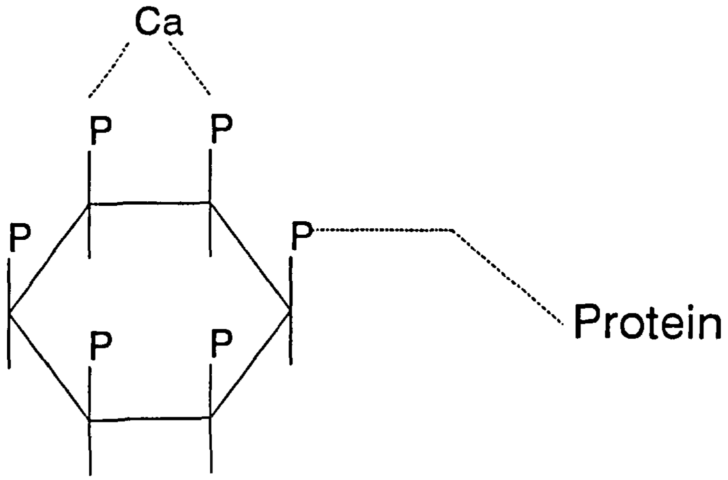

Ca
P
P
P
P
P
P
Protein

Figure 2.14 Rate of loss of phytate during autoclaving inositol hexaphosphate and natural products at 115°C (de Boland *et al.*, 1975)

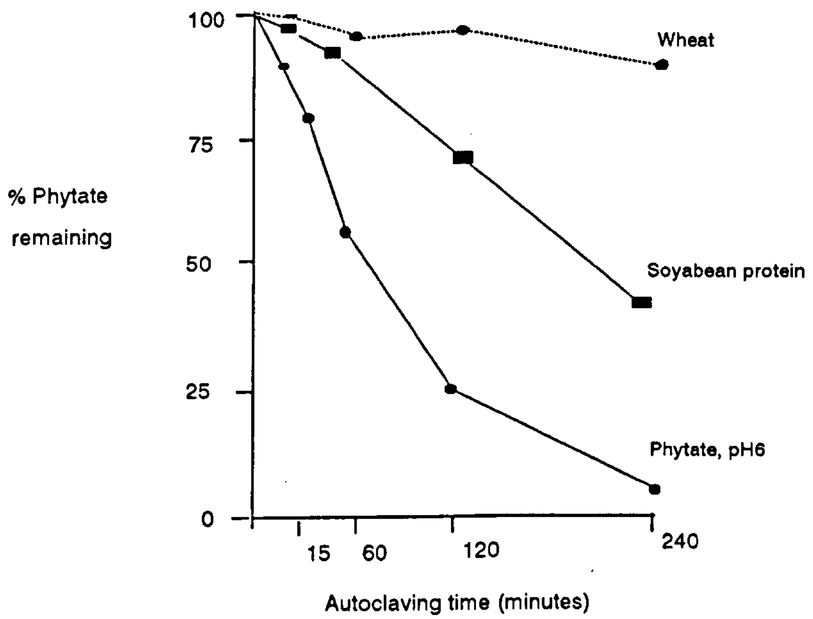


Table 2.9 Phosphorus content and availability in feedstuffs of animal origin

Feedstuff	Phosphorus (g/kg DM)	Phosphorus digestibility (%)
Meat meal	22.5	74
Meat meal	33.1	85
Bone meal	85.7	68
Fish meal	25.2	86
Hydrolysed feather meal	1.6	75
Skimmed milk powder	10.6	91
Whey powder	14.8	82
Bone precipitate	176.3	87
Meat and bone meal	87.6	80

Source: Jongbloed *et al.*, 1991

Table 2.10 Phosphorus content and relative availability of mineral phosphates

Mineral source	% phosphorus	decreasing availability
Ammonium polyphosphate	32	↓
Chloroapatite	17	
Curacao island phosphate	15	
Dicalcium phosphate	18	
Deflourinated phosphate	14	
Disodium phosphate	3	
Hostaphos	17	
Monoammonium phosphate	27	
Monocalcium phosphate	25	
Magnesium phosphate	19	
Monosodium phosphate	20	
Natural rock phosphate	13	
Phosphoric acid	28	
Soft phosphate	15	
Sodium pyrophosphate	23	
Sodium tripolyphosphate	25	
Superphosphate	15	
Tricalcium phosphate	20	
Trisodium phosphate	17	
Triple superphosphate	21	

Source: Jongbloed (1987)

2.4 Use of phytase to improve phytate-phosphorus availability

2.4.1 Mechanism of action

In its free form, phytic acid is unstable and undergoes hydrolysis. Myo-inositol hexaphosphate phosphohydrolase, or phytase, catalyses the dephosphorylation of phytic acid by stepwise removal of orthophosphates, resulting in the intermediates inositol mono to penta-phosphate (IP_1-IP_5) and free myo-inositol. Commercially available extrinsic phytases for pigs are defined as non-specific mono-esterases, belonging to the group of acid phosphatases. 2 classes are recognised: 6 phytase (EC 3.1.3.26) is specific for inositol hexaphosphate, initially hydrolysing orthophosphate from the 6-C position of phytic acid and thereafter splitting phosphoric acid from alternate carbon atoms, whereas 3 phytase (EC.3.1.3.8) initially removes orthophosphate from the 3 position of phytic acid. The mode of action is:

Myo-inositol hexakis phosphate (IP_6) ----------> D-myo-inositol 1,2,4,5,6 pentakisphosphate
+H_2O + orthophosphate (PO_4)

The reaction proceeds in a stepwise manner, producing the five classes of intermediate products; myo-inositol pentakis-,tetrakis-,tris-,bis- and monophosphates (IP_5,IP_4,IP_3,IP_2,IP_1 respectively).

2.4.2 Phytase of vegetable origin

Present in most feedstuffs of plant origin, phytase 6 activity is influenced by storage and processing but appears to retain some activity after ingestion. However, at a pH of less than 2.5 the enzyme is irreversibly inactivated therefore it is unlikely that plant phytase could survive the acid conditions of the stomach to resume activity in the small intestine. Lim and Tate (1971) studying the enzyme hydrolysis of wheatbran concluded that there are several pathways by which the penultimate myo-inositol-2-phosphate is produced. The major pentaphosphate formed is *D* myo-inositol

1,2,3,4,5, pentakis-phosphate which is also the major pentaphosphate formed from soybean phytase. Properties of soyabean phytase have been studied by Gibson and Ullah (1990). The pH optimum is 4.5-4.8, activity falling rapidly at pH 6 and above. This optimal pH is typical of many acid phosphatases as well as phytases (Nayini and Markakis,1986). The optimum temperature is 50°C, with denaturation occurring at 60°C. Although the enzyme shows specificity for phytic acid, a variety of phosphoesters can be used as substrates. The turnover rate is extremely slow which may be due to competitive end-product inhibition.

Attempts have been made to quantify the effect of plant phytase on phosphorus digestibility. The presence of phytase present in wheat enhanced phosphorus digestibility in wheat from 31-49% (Jongbloed and Kemme, 1990b). Lantzsch *et al.* (1992) reported that due to dietary plant phytase approximately 38% of phytate phosphorus from maize was absorbed in the stomach and the proximal half of the small intestine, whereas 55% was absorbed in the distal region of the small intestine.

2.4.3 Intestinal phytase activity

Whether or not phytase is secreted by the intestinal mucosal cells of monogastrics is subject to debate. Despite the differences in pH-activity and substrate-activity profiles between phytase and alkaline phosphatase (EC 3.1.3.1), their similar distributions, regional variations in activity and dependence on Mg^{2+} and Zn^{2+} for maximal activity have led to the suggestion that 'phytase' activity of the small intestine may be a manifestation of alkaline phosphatase activity (Davies and Flett, 1977). Alkaline phosphatase activity of the intestine is not due to a single enzyme but to a number of isoenzymes which are characterized by an ability to hydrolyse phosphate-ester linkages at pH values greater than 7, and it would seem likely that at least some of the isoenzymes which contribute to alkaline phosphatase activity may function as phytases. Morris and Ellis (1976) proposed that intestinal phytase was of little significance in aiding metal ion absorption because they would be rapidly hydrolysed to insoluble hydroxides at the pH of small intestine. However, Cooper and Gowing

(1983) suggested that as phytase was present at the mucosal surface, absorption of released metal ions could occur before the complexing reactions rendered them unavailable. Furthermore, it has been suggested (Ramakrishan and Bhandari, 1977) that intestinal phytase may play a role in phospho-inositide metabolism in the mucosal cells. Intestinal alkaline phosphatase and phytase activities are largely influenced by dietary composition and hormonal balance. Excess dietary calcium and magnesium decrease the activity of both enzymes, whereas vitamin D_3 increases activity (Mccuaig *et al.*, 1972). The effect of vitamin D *in vivo* may be enhanced by changes in calcium and phosphorus absorption in response to vitamin D.

In pigs, intestinal phytase activity was found to be negligible compared with alkaline phosphatase activity (Pointillart *et al.*, 1985) even when the phosphorus intake was very low and mostly in the form of phytate. In contrast, alkaline phosphatase activity of the duodenum was stimulated when pigs were fed a low phosphorus diet. There is some evidence to suggest that adaptation to phytase hydrolysis increases with age in pigs, but intestinal phytases and phosphatases are ineffective for phytate hydrolysis in balanced swine diets, probably because of the adverse effects of calcium, which at a ratio of 6:1 (Ca:phytate) or more reduces the mucosal activity (Bhandari, 1989).

2.4.4 Microbial phytase

When addition of phytate to human diets is prolonged, the initial negative calcium balance becomes positive (Walker,1951) possibly due to increased microbial production for phytate hydrolysis. The activity of microbial phytase in the digestive tract is governed by factors such as pH, source and concentration. Microbial hydrolysis of phytate is of negligible importance in the stomach or small intestine of rats, but occurs in the caecum and continues in the colon, so that the concentration of hydrolysis products is greatest in the faecal pellet (Wise *et al.*, 1983). The mechanism of hydrolysis is unknown, but it is possible either that bacteria employ a different mode of attack to that of mucosal phytase or that they have more time to perform the reaction on the digesta in the caecum than the mucosal enzyme.

Various strains of micro organisms produce phytase intracellularly; the most active extracellular sources are the filamentous fungi. In a study of micro organisms (Shieh and Ware, 1968) it was found that *Aspergillus niger* (var *ficuum*, NRRL 3135) produced maximal phytase. This phytase has been purified and characterised (Gibson and Ullah, 1990). *Aspergillus niger* secretes a phytase and 2 acid phosphatases representing approximately 8.5% of the total protein in the crude culture filtrate.

2.4.4.1 Properties of *Aspergillus niger* phytase

Aspergillus niger phytase is a glycoprotein with a molecular mass of 85-100 KDa. The enzyme is highly active and fairly thermostable; in its purified form retaining 40% of its activity after being subjected to a temperature of 68°C for 10 minutes. The temperature profile is asymmetric (fig. 2.15) with an optimal activity between 60 and 70°C, however the exact optimum may depend upon the type of cereal to which its added. Irreversible inactivation occurs at temperatures of 80°C or more.

2 distinct pH optima are shown (fig.2.16) The highest activity is at pH 5.0 and a second peak is observed at pH 2.5. The enzyme retains most of its activity at pH 6.0, but no activity is observed at pH 7.0 or higher. The *Aspergillus niger* phytases are

acid and heat resistant over a broad pH and temperature range (pH 2-6, and up to 60°C) Therefore, they may reasonably be expected to be active and to function in the stomach of pigs under physiological conditions.

2.4.4.2 Commercial manufacture of phytase

Present commercial development follows one of two procedures. The first involves conventional genetic improvement of existing phytase-producing micro organisms. The second approach entails isolating the gene controlling phytase production from a wild-type *Aspergillus* strain and splicing it into the genetic code of a hyper-producing industrial strain. The resultant increase in phytase production can be 50-200 times that observed from more classical techniques (Power, personal communication). For large-scale manufacture of the enzyme, fungal spore preparations are transferred through a series of batch fermentations, and the batch culture products used as inocula for final fermentation. Phytase production is maximal when phosphate is limiting ($<0.0004\%$ w/v).

2.4.4.3 Dephosphorylation of phytate

Phytase has a higher affinity for phytic acid than for other phosphorylated substrates, although it has a broad specificity towards other phosphorylated monoesters. *D*-myo-inositol 1,2,4,5,6 pentakis phosphate is the major component of hydrolysis, with a minor component of *D*-myo-inositol 1,2,3,4,5 pentakis-phosphate.

It is generally accepted that phytase can completely hydrolyse phytic acid to the monophosphate stage, and perhaps to free inositol and orthophosphate. However, it has been found that phytase from *Aspergillus* shows reduced turnover number with increasing incubation time (Gibson and Ullah, 1990) This may reflect lowered affinity of phytate for intermediate inositol forms; myo-inositol- 1-monophosphate was not utilised as a substrate and myo-inositol was only poorly utilised.

A non-purified preparation of intracellular acid phosphatase (EC 3.1.3.2) from a waste mycelium of *Aspergillus* niger was tested for enzymic hydrolysis of phytate compounds *in vitro*. Complete dephosphorylation of soybean protein isolates was achieved using 12 units per gramme of feed, this was accompanied by protein degradation which was imputed to proteolytic activity of the phytase as other proteolytic enzymes were inhibited during preparation (Zyla *et al.*, 1989). Phytase from *Aspergillus ficuum* (NRRL 3135) resulted in hydrolysis of 63% and 42% of the phytate of soybean meal and cottonseed meal respectively (Han, 1989), and indicated that phytate hydrolysis *in vivo* would be largely diet dependant. Thus far, no correlation has been found between extent of phytic acid degradation and total phosphorus digestibility. This requires quantification of intermediates and comparison of phosphorus digestibility with the amount of orthophosphate.

2.4.5 Use of phytase in animal feeds

2.4.5.1 Early work with poultry

Pioneering work using phytase in the diet was carried out by Nelson *et al.* (1968, 1971). A crude enzyme preparation from *Aspergillus ficuum* (NRRL 3.1.3.5.), consisting of a non-specific orthophosphoric monoester hydrolase and phytase, was supplemented into corn-soybean meal diets at levels of 1-8 g/kg feed. Performance was compared to that of chicks fed different levels of inorganic phosphate. The increase in percentage bone ash obtained when diets contained either 4 or 8g phytase/kg was equivalent to 0.16 and 0.17% of added inorganic phosphate; this represented total hydrolysis of phytate. Bone ash percentage of these chicks was higher than those fed the diets supplemented with inorganic phosphate to contain 0.24% available phosphorus, but less than that of chicks fed the diet containing 0.33% available phosphorus. Almost all the dietary phytate was hydrolysed with 3g/kg phytase.

Later, Zyla *et al.* (1989) supplemented calcium phosphate into diets in order to provide the same amount of phosphorus as that calculated to be released by phytase.

After 3 weeks, body weight of enzyme-fed chicks was significantly lower ($p < 0.05$) than those with the mineral supplement, however, after 8 weeks body weight was significantly greater ($p < 0.05$) when the enzyme was used. Feed conversion efficiency and tibia mineralization did not differ significantly between the groups.

A similar preparation of *Aspergillus* phytase significantly improved the apparent digestibility of phosphorus in broilers, with almost maximal phosphorus availability achieved by the addition of 800 units of phytase/kg feed (Simons *et al.*, 1990). Growth rate and feed conversion efficiency were significantly improved and dependent on the level of phytase; the optimum level for growth rate from 0-4 weeks was 1500 units. Increased phosphorus digestibility resulted in a decreased faecal phosphorus excretion; in addition, increased calcium retention was observed.

2.4.5.2 Use of phytase to improve phosphorus availability to pigs

Initial experiments to test the effect of phytase on phosphorus digestibility in pigs was carried out in the Netherlands (Simons *et al.*, 1990). Pigs were cannulated at two sites of the gut; approximately 180mm beyond the pylorus and 200mm proximal to the ileo-caecal junction. Experimental diets were based either on corn-soybean meal or tapioca-hominy feed. Addition of 1000 units of *Aspergillus niger* phytase per kg feed resulted in an increase of 9-42 percentage units ($p < 0.01$), indicating that approximately 50% of the phytate-phosphorus was made available for absorption.
It was presumed that phytase increased hydrolysis of phytate at the gut lumen and thus liberated ortho-phosphates could be absorbed across the gut membrane.

These results provided the basis for renewed interest in phytase research, particularly in countries where phosphorus levels were of growing concern. Recent published works are listed in table 2.11. In most studies, phytase was obtained by a strain of *Aspergillus*, which produces 3 - phytase (EC 3.1.3.8). Criteria of response included digestibility of phosphorus and other nutrients associated with phytase, bone breaking strength or carcase mineralization. Since the initial work, further experiments have indicated that if phytase is used correctly, up to a 50% reduction of faecal phosphorus output may be possible (Kessler and Egli, 1992; Pallauf *et al.*, 1992a).

Further work using cannulated pigs (Jongbloed *et al.*, 1991) showed that in the presence of phytase, 60-74% of phytic acid could be hydrolysed before reaching the end of the small intestine, whereas only 10% of phytic acid from unsupplemented diets was hydrolysed. Apparent ileal digestibility of phosphorus was increased by 18.5% ($p<0.05$) and 29.8% ($p<0.01$) for maize-based and tapioca-based diets respectively. Total tract phosphorus digestibility increased by 29.7% (Maize) and 27.0% (Tapioca). Concentration of myo-inositol pentakis and tetrakis phosphates was higher in ileal digesta than in duodenal digesta, and the amounts were related to phytate sources. No phytase activity could be detected in the ileal digesta, and it was concluded that phytate degradation occurred in the gastro-duodenum; subsequently, liberated orthophosphates were absorbed in the small intestine. Concentration of phosphorus in the faeces was reduced from 21 to 13.6g/kg when a corn-soy diet was fed, and from 15.8 to 10.4 g/kg with a tapioca-hominy diet.

These authors found lower values for phytic acid digestibility at the ileum compared to the duodenum, and postulated that this may be due partly to *de novo* synthesis of myo-inositol phosphates in the small intestine. However, while such synthesis has been observed in the blood of some species, there is no evidence to suggest that myo-inositols can be absorbed across the gut wall. Thus the decreased digestibility at the ileum may have been a manifestation of increased absorption at the duodenum compared to the ileum, as seen by Partridge (1978).

Further work showed that the response was also dependent on the concentration of phytase activity in the diet (Beers, 1992; Young *et al.*, 1993; Cromwell *et al.*, 1993). Although the shape of the response curve obtained differed between experiments, a common feature appeared to be a diminution of response as the dose level increased past approximately 400-500 units/kg.

Figure 2.15 Temperature-activity profile of *Aspergillus niger* phytase

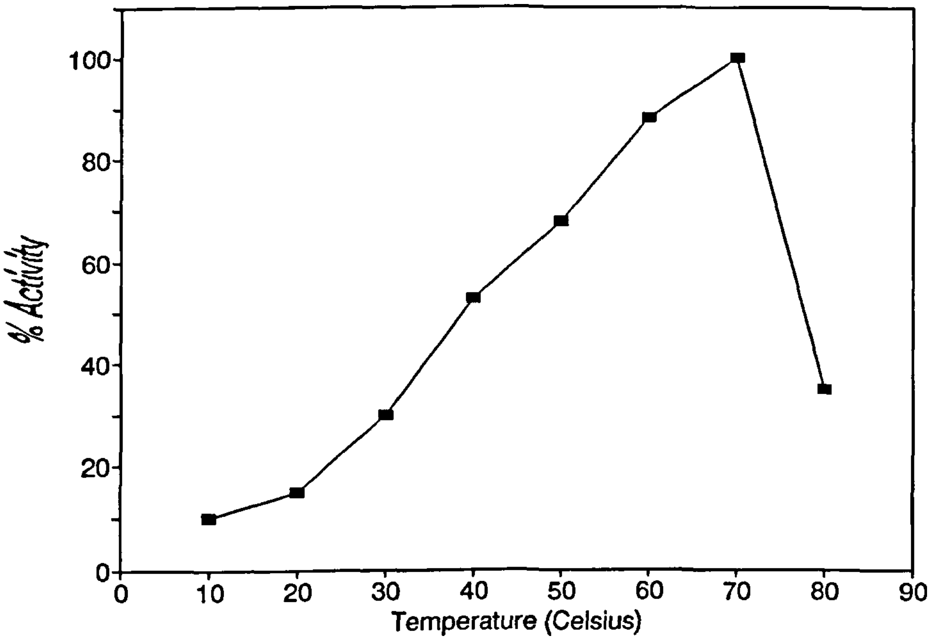


Figure 2.16 pH-activity profile of *Aspergillus niger* phytase

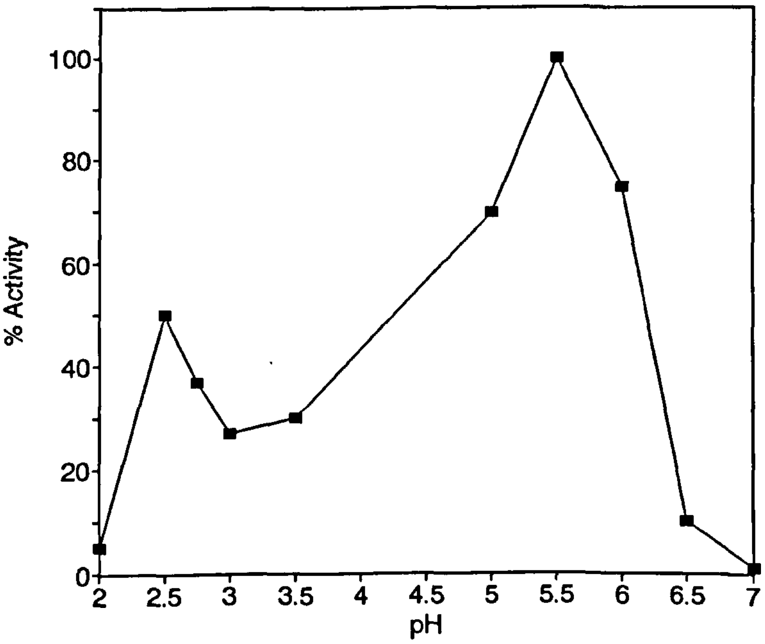


Table 2.11 Summary of recent phytase trials using growing pigs

Reference	Diet	Phytase activity (IU/kg feed)	Main results
Beers, 1992	maize, soybean + hominy, sunflower, rapeseed	220-1780	Exponential increase in P digestibility Logistic " "
Beers and Jongbloed, 1992	maize, barley, soya	1450	Increased P digestibility from 0.39 to 0.60 Improved DLWG, FI and FCR
Cromwell *et al.*, 1992	corn, soybean	250, 500, 1000	Increased P digestibility from 0.25 to 0.57 1/3 phytate-P converted to an available form
Cromwell *et al.*, 1993	corn, soybean	500, 1000, 2000, 4000	Reduced growth, FCR and bone strength caused by low dietary phosphorus counteracted by phytase
Dungelhoef *et al.*, 1994	maize wheat triticale	750	Increased P digestibility from 0.18 to 0.56 " " 0.62 to 0.74 " " 0.52 to 0.67
Eeckhout and de Paepe, 1992a	maize, soybean wheat, soybean	1000	Increased P digestibility from 35.6 to 54.7% " " 53.8 to 68.3% 30% reduction of faecal P excretion
Eeckhout and de Paepe, 1992b,c	maize, soybean + wheat middlings	250, 500	Microbial phytase 74% more efficient than wheat phytase 80% activity at pH of stomach contents (pH3) Wheat phytase totally inactive at similar pH Differences in transit time, gut pH values, and proteolytic activity between piglets and 50kg did not affect phytase efficacy
Hohler *et al.*, 1991	maize, soybean	500, 1000	Increased plasma Zinc concentration

Table 2.11 cont.

Reference	Diet	Phytase activity (IU/kg feed)	Main results
Hoppe *et al.*, 1993	cereal, soybean	125, 250, 500, 1000	380 units phytase equivalent to 1g inorganic phosphate Based on total body P retention
Jongbloed *et al.*, 1992	corn, soybean tapioca, hominy	1500	Increased ileal P digestibility from 26.4 to 44.9 " " 16.0 to 45.8
Kemme and Jongbloed, 1993	maize, tapioca, peas, potato protein, soybean	500	Phytase efficacy not dependent on phytate in feeds Soaking increased effect of phytase
Kessler and Egli, 1992	liquid feed	500	Decreased urinary plus faecal phosphorus output by 50%
Ketaren *et al.*, 1993b	sugar, soybean	1000	Increased gain from 741 to 835 g/day Improved FCR from 2.37 to 2.16 Increased protein deposition rate from 108 to 123 g/day
Lantzsch and Wjst, 1992	barley, soybean, sunflower meal	1000	Increase in digestible calcium of between 0.14-0.79 g/kg
Lei *et al.*, 1993a	maize, soyabean	250, 500, 750	Linear increase in DLWG and DFI Linear increase in plasma inorganic phosphate Linear decrease in plasma alkaline phosphatase
Lei *et al.*, 1993b	maize, soybean	750, 1050, 1250, 1350	Quadratic response of DLWG, FI and FCE " plasma alkaline phosphatase Stationary points at 1200 units/kg 1000 units of phytase supported retention of 1.1mg P ≡ 0.91mg P from mono-dibasic calcium phosphate

Table 2.11 cont.

Reference	Diet	Phytase activity (IU/kg feed)	Main results
Lei *et al.*, 1993c	maize, soybean	1350	Phytase or Zn increased plasma Zn and alkaline phosphatase Phytase plus Zn decreased plasma alkaline phosphatase activity
Mroz *et al.*, 1991	maize, tapioca, soybean	800	Increased ileal digestibility of methionine, cystine, arginine, isoleucine and phenylalanine
Mroz *et al.*, 1994	maize, tapioca, barley, soybean	300 600	Liberated 0.6g digestible P from phytate " 0.91g
Nasï and Helander, 1994	barley, soybean	1200	No effect of soaking on digestibility Increased P digestibility from 0.45 to 0.64
Pallauf *et al.*, 1992a	maize, soybean	1000	Phytase could replace 0.2% inorganic P supplements Reduced faecal P excretion by 50%
Pallauf *et al.*, 1992b	maize, soybean	500 1000	Increased Mg, Zn, Fe and Cu absorption, Mn unchanged 1000 U further improved Zn
Pallauf *et al.*, 1994	Faba beans, wheat, peas, barley	350 700	Increased P digestibility from 0.48 to 0.66 " " 0.48 to 0.71 Small increase in OM and DM digestibility with 700U
Simons *et al.*, 1990	maize, soybean tapioca, hominy feed	1000	Increased P digestibility from 0.20 to 0.46 " " 0.34 to 0.56
Simoes Nunes 1994	maize, rapeseed, barley	200	Reduced faecal P excretion by 25%
Young *et al.*, 1993	Corn, soybean, canola'	500, 1000	Increased ash and phosphorus of metatarsal Response equivalent to 1.7gP from calcium phosphate

2.4.5.3 Phosphorus equivalence of phytase

If most of the phosphorus present in cereals fed to pigs was made available for digestion, it should be sufficient to satisfy the requirement of growing pigs. Thus far, few experiments have addressed the question of phosphorus/ phytase equivalence. Using the slope-ratio method based and a range of criteria such as growth performance, mineral content of the body and bone ash, a low-phosphorus cereal-soybean meal diet was supplemented with increasing phytase up to 1000 units/kg (Hoppe and Schwarz, 1993). Based on phosphorus retention in the total body and bone ash it was calculated that 1g of inorganic phosphate from monocalcium phosphate was equivalent to 380-400 units of phytase. Lei *et al.* (1993a) based their response on growth performance and plasma alkaline phosphatase concentration of weanling pigs. 1000 units of phytase supported retention of 1.1mg P from a corn-soy diet, and was equivalent to 0.91mg from monodicalcium phosphate. The substantial difference in these two estimates indicate the need for further research in this area before phytase can be confidently substituted for inorganic phosphorus.

2.4.5.4 Effect of phytase on digestibility of other minerals

As previously described in *Section 2.3*, phytate complexes may be formed between phytic acid and other minerals, such as calcium, magnesium and trace minerals. During degradation of phytates by hydrolysis it is feasible that these minerals may be liberated and their digestibility improved. Increases of between 7 and 17 percentage units in calcium digestibility in response to phytase were reported in pigs of 20-25kg liveweight (Lantzsch and Wjst, 1992). In a series of experiments at the IVVO DLO in the Netherlands (Jongbloed *et al.*, 1993), phytase supplementation to a range of dietary sources resulted in increases in digestible calcium of between 0.14 and 0.79 g/kg. Further work showed that the increase in calcium digestibility was related to the dietary level of calcium, with the maximal increase (10.8 percentage units) obtained at the lowest level of calcium (4g/kg; Mroz *et al.*, 1993).

Little information exists about the effects of phytase on trace mineral availability.

Studies of Pallauf *et al.* (1992) showed that apparent digestibility of magnesium, zinc, copper and iron increased by 8-13, 7-13, 3-7 and 2-9 percentage units, respectively. Conversely, no effect of phytase on plasma magnesium, iron or copper concentration were found by Lei *et al.* (1993c) although plasma zinc concentration was increased.

2.4.5.5 Effect of phytase on digestibility of protein and amino acids

Interactions occurring between phytate and proteins in the digestive tract prevent the degradation of proteins by proteolysis, resulting in a decreased protein digestibility. By disrupting the phytate molecule during hydrolysis, phytase may cause phytate-protein bonds to be cleaved, allowing liberation of proteins for absorption. Proteases that may be present in the enzyme preparation may also exert an effect.

No effect of phytase on apparent total tract digestibility of nitrogen was observed by Nasï (1991). Other workers found an improved apparent digestibility of ileal nitrogen ($p < 0.01$) and increased nitrogen retention when 800 units/kg phytase were used (Mroz *et al.*, 1991). Increased apparent ileal digestibility of methionine, arginine, ($p < 0.01$), cystine, isoleucine and phenylalanine ($p < 0.05$) was observed with addition of the enzyme. It was postulated that while the increased digestibility of individual amino acids may have reflected their increased availability for absorption, the increased luminal pool of orthophosphates may also have enhanced energy metabolism at the cellular level, thereby increasing active transport of amino acids across the basolateral membrane. Increases in digestibility of amino acids in this experiment were in the order of 3-6 percentage units, in contrast to later results where increases of 7-12 percentage unit increases in ileal digestibility of essential amino acids were observed (Officer and Batterham, 1992).

No improvement of nitrogen digestibility was detected when phytase was added to grower pig diets (Ketaren *et al.*, 1991) although protein deposition rate was significantly increased with phytase (123 vs 108 g/day). One explanation was that the diets were so deficient in phosphorus that protein deposition was limited; this was overcome by the release of phosphorus caused by phytase.

2.4.5.6 Effect of phytase on growth performance

Replacement of inorganic phosphates by phytase is possible only if growth is not compromised. Having established repeatable improvements in phosphorus digestibility, attention has more recently turned towards growth performance. Addition of 1450 phytase units/kg to a diet containing 1.6g/kg digestible phosphorus (4.2g/kg total phosphorus) resulted in an improved feed intake (802 vs 695 g/day; $p<0.05$), daily liveweight gain (529 vs 424 g/day; $p<0.05$) and feed conversion ratio of piglets (1.52 vs 1.65; $p<0.05$; Beers and Jongbloed, 1992). Similar improvements in feed intake (0.89 vs 0.81 kg/day), daily liveweight gain (0.54 vs 0.45) and FCR (1.65 vs 1.8) were found by Young *et al.* (1993) when 1000 units of phytase/kg were used in a piglet diet containing 5.7g/kg total phosphorus.

No effect of phytase on carcase composition has so far been reported, although as previously described, increased protein deposition rate of pigs growing from 20kg-50kg liveweight was seen in response to phytase (Ketaren *et al.*, 1993). In this experiment, improved growth rate (835 vs 741 g/day; $P<0.05$) and feed conversion ratio (2.16 vs 2.37; $P<0.01$) of pigs receiving phytase were also observed. In another experiment, addition of phytase partially overcame a reduced growth performance caused by low phosphorus (Cromwell *et al.*, 1993). Growth of pigs receiving the 3g/kg phosphorus supplemented with phytase was 790 g/day, compared with 853 g/day of pigs receiving 5g/kg phosphorus.

The improved growth performance seen when phytase is added to phosphorus deficient diets has been associated with the re-establishment of normophosphataemia. When 2000 units/kg phytase were added to a phosphorus - deficient diet based on maize, rapeseed and barley diet fed to growing pigs, improved liveweight gain (505 vs 414 g/day; $p<0.05$) was accompanied by an increase in blood phosphorus from 2.04 to 2.97 mmol/litre; Simoes -Nunes, 1994).

2.4.5.7 Practical considerations of phytase application

The concept of using microbially-derived phytase to improve phytate-phosphorus availability is not a new one. However, application of the concept to diets of growing pigs has only relatively recently been realised, due to technological difficulties and economic concerns which still partly preclude its widespread use. More recently, improvements in microbial strain and efficiency have overcome some of the cost constraints. The main existing problem is the severe loss in activity at high processing temperatures. Pelleting of pig feeds is carried out in order to reduce dust and spillage, and for a possible improvement in digestibility. While phytase is stable up to 60°C, beyond this temperature, a dramatic loss in activity is observed. The feed industry is thus faced with the problem of retaining biological activity of enzymes applied to compound feed. One solution that has recently been adopted is application of the liquid enzyme after pelleting. Another option which extends from this is the use of phytase in liquid feeds. Phytase increased the phosphorus digestibility of a phytate-rich diet from 0.27 to 0.44; when the diet was soaked for 8-15 hours prior to feeding, phosphorus digestibility increased further to 0.52 (Kemme and Jongbloed, 1993).

In vitro studies of Hans and Wilfred (1988) indicated that hydrolysis of phytic acid in feedstuffs was related to incubation time. This would suggest that in practise, feeding frequency and level may reflect on the effectiveness of the supplemented microbial phytase. In growing pigs, plane of nutrition (2.3 or 2.8 x maintenance energy requirement) had little influence on the effect of phytase. However, feeding frequency influenced the apparent ileal digestibility of phytate (Mroz *et al.*, 1994). In contrast, feeding frequency and level influenced phytase activity only to a minor extent in piglets (Kemme and Jongbloed, 1994). In spite of these differences, it appears that there is little interaction between phytase activity and age; thus the possibility exists to use phytase in the diet of breeding stock and piglets, as well as growing and finishing pigs.

2.5 Overview and introduction to experimental work

Large amounts of microbially derived phytase can now be commercially produced, bringing it closer to economic feasibility as an alternative to inorganic phosphate. Perhaps the most meaningful aspect of work to date is the indication that performance can be maintained even in the absence of phosphate supplements. One could anticipate that if used correctly, phytase could largely replace inorganic phosphate, particularly in regions where soil phosphate levels sources are of concern.

Despite the increase in phytase research over the past few years, and the general acceptance of the enzyme as a tool for reducing phosphate outputs, some key areas of research remain unsolved. Variation in responses obtained between different groups of workers demonstrate the lack of standardisation across experiments. Basic design elements have not always been followed and the phytase has not always been applied correctly. For example, uniform distribution of the enzyme within the feed is difficult to ensure but is a prerequisite to valid experimental data. Underlying the differences in results obtained from many of the experiments is the lack of consensus about the phosphorus requirement of growing pigs, due to many of the reasons already discussed. Finally, few data exist which allow commercial evaluation of phytase. Calculations of the amount of inorganic phosphate that can be replaced by the enzyme, and of the optimum inclusion level of phytase remain vague.

These considerations laid the foundation of this research programme. A series of trials was undertaken to assess the potential of phytase; its effect on digestibility of phosphorus and other nutrients, optimum inclusion level, and effect on growth and bone development of growing pigs. An extensive range of response criteria, including apparent ileal and total tract digestibility, growth performance, carcase composition, and bone strength and mineralization, were used to establish the phosphorus requirement of growing pigs, to assess the effect of phytase on phytate -phosphorus availability, and finally, to establish the net effect of replacing inorganic phosphate with phytase in the diet of growing pigs.

Chapter 3 General materials and methods

3.1 Introduction

This chapter describes the general materials and methods implemented during the experimental work. A total of four trials were carried out; two of which were metabolism trials (one involving cannulated pigs) and two growth trials. The metabolism studies were performed in order to look at the effect of *Aspergillus niger* phytase and *Saccharomyces cerevisiae* yeast on apparent ileal and total tract digestibility of phosphorus, and to determine an optimum dietary inclusion level of phytase, using total tract digestibility of phosphorus in growing pigs (25-60kg) as the criterion of response. The growth trials were undertaken in order to establish a dietary phosphorus requirement and to assess the effect of phytase on phosphorus availability using a range of criteria including growth, carcase composition, bone strength and bone mineralization as indicators of requirement and availability in young pigs weighing 10-25 kg and in growing pigs weighing 25-60kg.

3.2 Experimental diets

The experimental diets were formulated to contain low levels of phosphorus of which a high proportion was present as phytate-phosphorus, in order to maximize any response to the enzyme. Values of phytate-phosphorus were obtained from published tables (Jongbloed, 1987; table 3.1). In all experiments a basal ration was produced and the appropriate amount of enzyme was then added. For the first experiment involving cannulated animals, powdered titanium dioxide was mixed into the basal ration at a level of 1g/kg as a dietary marker. With the growth trials, increasing levels of phosphorus were achieved by the addition of mono-ammonium phosphate. With the exception of phosphorus, all nutrients were included at or in excess of the ARC (1981) recommended levels.

3.2.1 Preparation and testing of the phytase

The enzyme used was prepared by Alltech Biotechnolgy Inc. using fermentation of a strain of *Aspergillus niger* (var *ficuum*; NRRL 3135). The culture medium consisted of corn starch, sodium nitrate, magnesium sulphate, potassium chloride, ferric sulphate and sodium phytate. The dried preparation, containing fungal phytase and other enzyme secretions, was packaged in polythene and stored at -4°C. Specific activity of each enzyme batch was determined prior to incorporation into the diet, using an assay described in *Section 3.6.7.* Specific activity was determined at the University of Nottingham and verified at the University of Galway (Galway, Ireland). The acceptable variance was 5%. A 5% loss in activity was expected over 1 year under normal storage conditions.

3.2.2 Preparation and testing of the yeast

The yeast product was prepared by Alltech Inc. Live *Saccharomyces cerevisiae* (NCYC^{1026}) were grown on a medium composed of ground corn, malt and cane molasses. The yeast cells were harvested and tunnel-dried. Viability was tested at the point of production (Alltech Biotechnology Center, Kentucky, USA) before incorporation into the diet. The final product, containing 5×10^9 organisms/gram and some growth medium, was stored in polythene at -4°C.

3.2.3 Preparation of experimental diets

The basal feed ingredients were ground in a hammer mill (screen size 3.5mm) and then mixed in a one-tonne capacity vertical mixer. The diets were bagged into 25kg lots using conventional feed bags, and kept in a cool feed store until time of use. For the metabolism trials, titanium dioxide, yeast and phytase were added to the appropriate diets and mixed in a concrete mixer in 10kg batches prior to feeding. For the growth trials this was not practical as much larger quantities of feed were needed, thus the enzyme was added at the mill to the appropriate diets, along with the relevant amount of mono-ammonium phosphate.

3.2.4 Sampling of diets

Sampling of the diets was carried out throughout each experiment. Two samples each weighing approximately 200g were taken from representative bags. The samples were bulked and a sub-sample taken and stored at 5°C for laboratory analysis.

3.3 Experimental animals

The pigs used in these experiments were progeny of British Landrace boars backcrossed to British Landrace x Large White sows, supplied from the University of Nottingham pig herd. Health status of the animals was generally high; any illnesses encountered were dealt with according to veterinary and Home Office recommendations. Piglets were ear notched for identification, had their 'eye' teeth and tails cut and received an intra-muscular injection of an iron dextran solution at birth. Piglets were weaned at approximately 4 weeks of age and received a pelleted creep feed. After 4-5 weeks they entered the grower house where they were offered a standard grower diet *ad libitum*.

3.4 Housing of experimental animals

3.4.1 Pig metabolism trials

Prior to and between collection periods, animals were housed separately in holding pens containing a feeding trough and drinker. Ambient temperature was maintained at 18-22°C by thermostatically controlled fan heaters, and extractor fans were used to maintain airflow. Illumination was provided constantly in accordance with Home Office regulations for metabolism trials. Pens were cleaned daily. During collection periods the pigs were kept in adjustable metabolism crates with pvc-coated meshed floors which allowed drainage of water during washing of the cannula (figure 3.1).

3.4.2 Pig growth trials

For the first growth trial using young pigs, the animals were kept in separate holding pens of similar design to those used in the metabolism trial. Ambient temperature was 20-25°C and extractor fans operated continuously. During the second growth trial the pigs were accommodated in larger holding pens with concrete floors. Ventilation was provided by extractor fans, and gas heaters were used to achieve an ambient temperature of 18-22°C.

3.5 Experimental procedure

3.5.1 Cannulation experiment

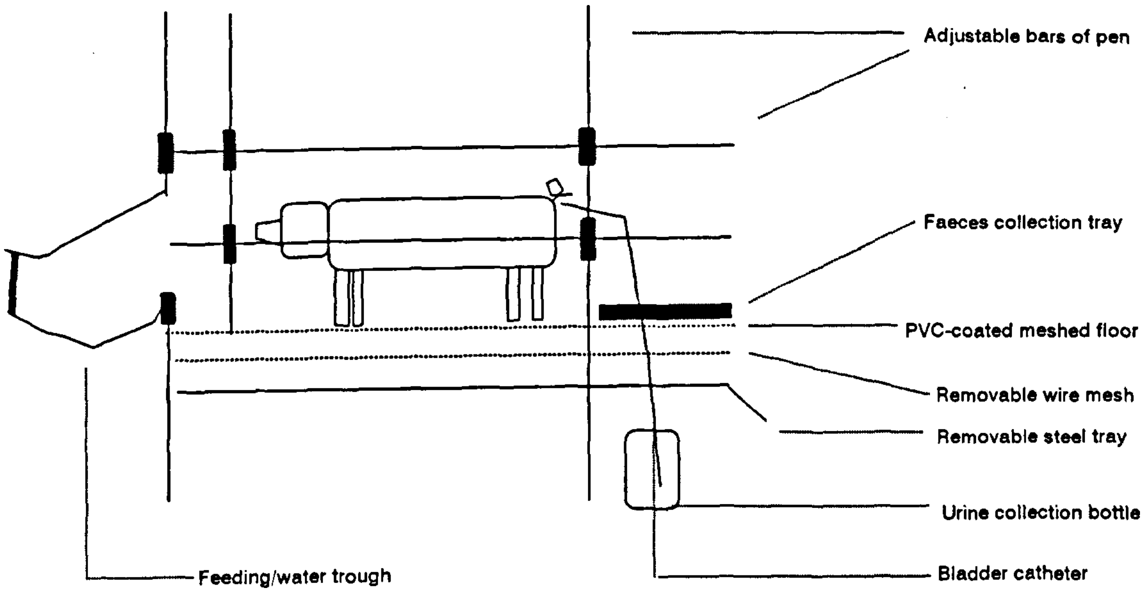
3.5.1.1 Preliminary treatment of animals

Female pigs weighing approximately 25kg liveweight were selected from the grower house and transferred to individual holding pens. 500g of the standard pig grower diet were offered twice daily; to this was added water in a ratio of 2:1 (weight water: weight feed). After 3 days all pigs were injected with 1ml of the antibiotic Tribrissen^R 48% (Trimethoprim + Sulphadiazine) before morning feeding; this treatment was continued for the next 2 days in preparation for surgery. Animals were starved for 24 hours preceding surgery, during which time water was offered *ad libitum*.

Table 3.1 **Phosphorus and phytate-phosphorus content of dietary ingredients**

Ingredient	Phosphorus g/kg	Phytate P g/kg
Barley	3.9	2.5
Maize	2.6	1.7
Soya 50	6.8	4.2
Rapeseed meal	6.7	2.3
Spring peas	4.3	2.2
Wheat	3.3	2.4
Oatfeed	-	-
Dicalcium phosphate	180	-
Dried skim	10.0	-
Whey powder	6.6	-
Betamix 314	-	-
Fish 66	33	-
Fat premix	2.0	-

Figure 3.1 Design of metabolism crates


Adjustable bars of pen
Faeces collection tray
PVC-coated meshed floor
Removable wire mesh
Removable steel tray
Urine collection bottle
Bladder catheter
Feeding/water trough

3.5.1.2 Surgical procedure

The pigs, now weighing approximately 35kg liveweight were transferred to the surgery area and washed. Each was sedated with an intra-muscular injection of Stresnil^{R} at a rate of 4mg/kg followed 30 minutes later by an intravenous injection of Thiopentone at a rate of 1.5mg/kg. The vocal cords were sprayed with a 2% lignocaine solution. An endotracheal tube was inserted, through which a halothane/ nitrous oxide/ oxygen mixture was administered. The right side of the animal was shaved, washed with antiseptic solution and wiped with chlorohexidine.

The animal was secured to the operating table and a subcostal incision was made approximately 2cm from the last rib, through which the caecum and terminal ileum were expressed. A purse string suture was made in the terminal ileum, approximately 10cm from the caecum, and the sterilised cannula inserted through an incision and exteriorised through a stab wound below the incision line. Thereafter the cannula was assembled and the peritoneum, muscle, subcutaneous tissue and skin were closed using iodine spray dressing between the skin layers.

3.5.1.3 Post-operative care of animals

Immediately after cannulation 0.7ml of Finadyne analgesic was administered intra-muscularly, and this was continued for 2 days after surgery. Tribrissen treatment was continued as previously described for a period of 4 days post-operatively. After recovery from anaesthesia, pigs were transferred to their holding pens where the ambient temperature was increased to 22-24°C. Water only was provided on the day of surgery; over the next two days up to 1 litre of milk replacer (Denkavit) was offered twice daily, and for the subsequent two days increasing amounts of the basal experimental diet were substituted into the meals. Thereafter, the amount of feed offered was gradually increased to 1200 grammes per day.

Every day throughout the trial the cannula and surrounding skin were washed with a diluted antiseptic solution and covered with talcum powder. The experimental period commenced 18 days after cannulation by which time the animals were readily consuming the feed offered. The trial then proceeded as described in *Section 3.5.2.*

3.5.2 Pig metabolism trials

3.5.2.1 Acclimatization

Upon arrival at the metabolism building the gilts were weighed and transferred to holding pens, and introduced immediately to twice daily feeding with the standard pig grower diet. The feeding scale used was that derived by Morgan (1972) based on the equation $y = 0.816 + 0.021x$ (where y = kg DM feed/day; x = kg bodyweight; table 3.2). Animals were fed daily at 0800 hrs and 1600 hrs, with access at feeding time limited to 30 minutes per meal.

A summary of the procedure followed throughout the metabolism trials is given in appendix I. Following successful adaptation to the feeding conditions, each pig was assigned randomly to one of the experimental diets for a preliminary 10 day acclimatization period. On day 8 of the adaptation period, pigs were weighed and transferred to metabolism crates, in order to adapt them to the conditions inside the crate prior to the commencement of collection. An indwelling bladder catheter (Warne surgical products) was inserted into each pig to facilitate urine collection. The size of the catheter used depended on the liveweight of the pig; 14 gauge (5-15ml) was used for the first two collection periods and thereafter 16 gauge (10-15ml) was used. The catheter and the area around the vulva were disinfected with a dilute solution of Savlon, the catheter was inserted and the balloon inflated with sterile water injected through a tube running outside the catheter tube. Each catheter was connected to a polythene tube fastened to the side of the metabolism crate. The free end of the tube was inserted into a large urine bottle positioned at the rear underneath the crate.

Table 3.2 Dailly feeding allowance of pigs during metabolism trials

Liveweight (kg)	Feed (kg/day)
30-35	1.425
35-40	1.550
40-45	1.670
45-50	1.790
50-55	1.895
55-60	2.000
60-65	2.100
65-70	2.195

Figure 3.2 Structure of experimental periods (cannulation experiment)

Adaptation	Faeces and urine collection	Ileal collection
10 days	5 days	5 days

3.5.2.2 Faeces and urine collection

Total faecal output was collected over a 5 day period using a marker-to marker technique. On day 10 of the adaptation period powdered indigo carmine dye was mixed into the afternoon feed at a level of 5g/kg. Collection of faeces commenced with the appearance of the dye in the faeces of each pig. A sheet of plyboard was fitted to the floor of the metabolism crate behind each animal onto which most of the faeces was deposited. Any faeces that was not deposited onto the board fell through the meshed floor and was collected from the underlying metal tray. Faecal output was collected twice daily (at feeding), accumulated in polythene bags and stored at -18°C. At the end of the collection period, faeces for each animal was thawed, weighed accurately and homogenised for 15 minutes in a Hobarts baker's mixer. Duplicate subsamples (weighing approximately 150g) were collected into plastic cups and freeze-dried for analysis. The remainder of the faeces was discarded.

Urine collection commenced at 0900 hours on the day following the addition of the dye into the feed, and continued for 5 days, during which total urinary output was collected into the urine bottle containing 25ml of 50% sulphuric acid. Total urine output was weighed each morning and a 1% aliquot was transferred into a plastic beaker. The remaining urine was discarded, and the empty urine bottle was replenished with 25ml sulphuric acid. Samples of the urine from all 5 days were pooled and stored at -18°C.

On the 5th collection day, indigo carmine was again introduced into the afternoon meal, and collection was terminated as soon as the dye appeared in the faeces. In the first metabolism trial where cannulated pigs were used, the animals remained in the metabolism crates for a further 5 day ileal sampling period. In the second metabolism trial where total tract digestibility only was being measured, the pigs were weighed and transferred to the holding pens. The feeding level was adjusted according to the equation and the diet was changed according to the latin square design. A 10-day adaptation period was followed by the 5-day total collection as in period 1. This was repeated for periods 3 and 4.

3.5.2.3 Ileal sampling

Ileal sampling took place on alternate days during the 5-day collection period ie days 1,3 and 5. Ileal digesta samples were collected at 0, 4, 8, and 12 hours after the morning feed through the T-cannula barrel after releasing the plug. Samples were frozen immediately, freeze-dried and pooled for each animal, then ground finely and subsampled for analysis.

At the end of the collection period, the pigs were weighed and transferred to holding pens. The feeding level was adjusted according to the equation and the diet changed. The adaptation period of 10 days was followed by faecal and ileal collections as in period 1 (figure 3.2). This procedure was repeated for period 3.

3.5.3 Pig growth trials

3.5.3.1 Growth performance measurements

The first growth trial was carried out using 72 weaner piglets (36 male, 36 female) selected from the University pig herd at between 6-10kg liveweight. The piglets were transported to the experimental building where they were fed the standard grower feed *ad libitum*. All animals were weighed weekly; in addition those nearing the start or finish weight were weighed between weekly weighings. Each animal was assigned to its experimental diet when it reached 10kg liveweight, or on the first weighing subsequent to this, and each animal was deemed to have finished the trial when it reached 25kg liveweight or at the first subsequent weighing. During this time, sufficient feed was always available in each trough to satisfy the appetite of the corresponding pig. Fresh feed was offered every morning, and troughs were checked again in the evening, and replenished where necessary. Troughs were emptied weekly and any stale feed discarded. Feed intakes were recorded daily.

In order to dispense with gender variation, the second growth trial used 72 boars, which were selected at weights between 18-26kg. The standard grower diet was fed until the pigs reached 25kg or the first subsequent weighing, thereafter the experimental diet was introduced and maintained until the pig reached 60kg or the first weighing after 60kg. The feeding procedure followed was the same as for the first growth trial.

3.5.3.2 Slaughter of the animals and collection of samples

On reaching the predetermined slaughter weight, pigs were starved for 24 hours preceding slaughter; then reweighed to obtain the starved liveweight, slap-marked and transferred to the University's slaughter house where they were killed by electrical stunning and ex-sanguination. For the first trial analysis of the whole body was required, thus blood was collected into a polythene bag, and weighed. The alimentary canal was removed and weighed full, then cleaned, dried and weighed empty. The empty carcase was then weighed.

The right hind femur and the right third and fourth metatarsal bones were removed and sealed in individual plastic bags. Bones were stored frozen at -18°C. The empty carcase, blood and digestive tract were frozen prior to grinding in a Wolfking Mincer. Each pig was passed 3 times through the mincer to ensure thorough grinding, this also achieved sufficient mixing of the carcase to enable representative samples to be taken. Samples of approximately 150g were collected in duplicate into polythene pots and freeze-dried for laboratory analysis.

3.5.3.3 Carcase measurements

Pigs from the second growth trial were not used for whole-body analysis, and thus after slaughter conventional abattoir practises were followed. Carcases were chilled at 3°C for approximately 24 hours after slaughter and then weighed. The following linear carcase measurements were taken from the left side of the carcase:

1 Length of the carcase - measured from the anterior edge of the *symphysis pubis* to the vascular impression on the anterior edge of the first rib while the carcase was hanging (figure 3.3)

2 Depth of the backfat plus the subcutaneous layer -

at its widest in the region of the shoulder (maximum shoulder)

at its thinnest in the region of the mid-back (minimum midback)

at its thinnest over the middle of the exposed cross-section of the *m. gluteus medius* (minimum gluteus medius)

at its thickest in the region of the loin at the anterior edge of the *m. gluteus medius* (anterior gluteus medius)

at its thickest in the region of the loin at the posterior edge of the *m. gluteus medius* (posterior gluteus medius)

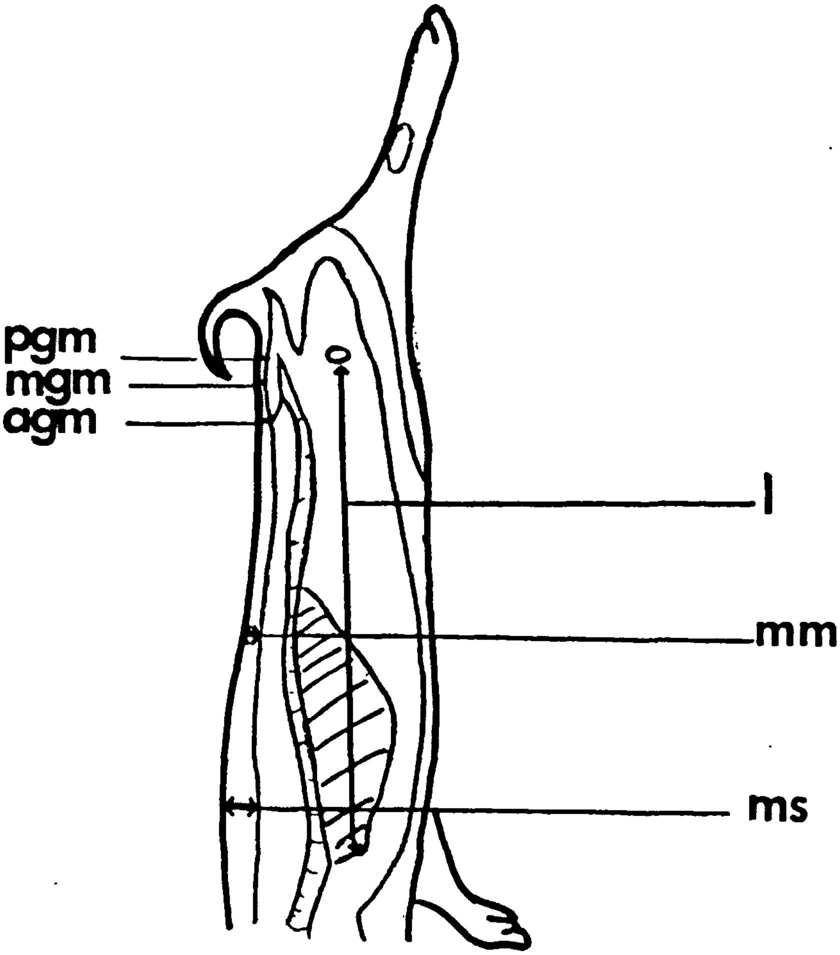
3 Fat deposition at P1, P2, P3, - measured on the cut surface of the last rib (figure 3.4)

4 Degree of lean and fat deposition measured around the *longissimus dorsi* muscle of the carcase quartered at the last rib (figure 3.4);

A The greatest width of the muscle cross-section
B The greatest depth of the muscle cross-section at right-angles to A
C The depth of subcutaneous fat plus skin directly over B

Following the carcase measurements the left third and fourth metatarsals were removed, separated and sealed in polythene bags for storage at -20°C.

Figure 3.3 Linear carcase measurements taken during *Experiment 3*



l = length of carcase

mm = mimimum midback

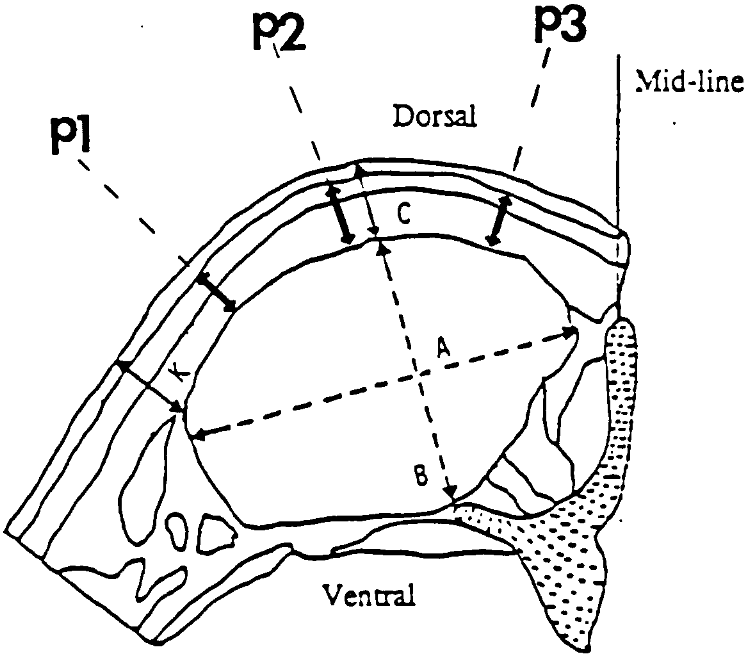
ms = maximum shoulder

pgm = posterior *gluteus medius*

mgm = minimum *gluteus medius*

agm = anterior *gluteus medius*

Figure 3.4 Measurements taken on the cut surface of the *Longissimus dorsi* muscle during *Experiment 3*


P2
P3
P1
Dorsal
Mid-line
C
A
K
B
Ventral

3.6 Laboratory analysis

Throughout the project, conventional analytical techniques were employed with slight modifications where appropriate. Analysis of samples of feed, enzyme, faeces, urine, ileal digesta, ground whole body and bone were in duplicate. The upper limit of variation was 5% beyond which the analysis was repeated in duplicate.

3.6.1 Proximate analysis

Freeze-drying of ileal digesta and faeces was achieved by transferring the frozen samples to a freeze-dryer for 10 days (digesta) or 7 days (faeces) during which a constant dry weight was achieved. Samples of the diets, freeze-dried ileal digesta and freeze-dried faeces from the first metabolism trial were analyzed for proximate components (dry matter, ether extract, crude fibre, ash and crude protein; N x 6.25) according to the methods outlined by the Association of Analytical chemists (AOAC, 1980). Samples of diet from the second trial were likewise subjected to proximate analysis. Freeze-dried faeces from the second metabolism trial were analyzed for dry matter, ash and crude protein (N x 6.25).

Feed samples from the growth trials were not analyzed for proximate components as digestibility of these nutrients was not being measured.

3.6.2 Gross energy determination

A Gallenkamp ballistic bomb calorimeter was used to determine gross energy of duplicate subsamples of feed, dried faeces and ileal digesta. Gross energy of the diets was confirmed using a Parr 1241 adiabatic oxygen bomb calorimeter.

3.6.3 Titanium analysis

Absorbance of titanium was measured at 408nm against standards prepared for a 0.1mg TiO_2/ml solution using a Pye Unicam Ultra-violet spectrophotometer which was blanked using a 10% solution of sulphuric acid (Jagger, 1987).

3.6.4 Mineral analysis

3.6.4.1 Preparation of samples for mineral analysis

Phosphorus and calcium analysis of all diets from the four trials and from each batch of phytase used throughout the trials was carried out. Phosphorus and calcium analysis of faeces and urine was carried out for the metabolism experiments. In addition, phosphorus and calcium content of the yeast and of the ileal digesta was measured in experiment 1, and of the samples of the ground whole body in experiment 3. The standard AOAC (1980) method of determination for organic materials was followed, using approximately 2g of sample weighed out accurately.

3.6.4.2 Preparation of urine samples

The level of phosphorus in the diet was low compared to the animal's requirement and consequently a low concentration of phosphorus was expected in the urine. To avoid being unable to detect the urinary phosphorus, approximately 40g of fresh urine was used for phosphorus analysis. The urine was weighed out accurately into a glass beaker, and the standard AOAC technique followed.

3.6.4.3 Preparation of bone samples

Following fat extraction as described in *Section 3.6.6*, the whole bone was ashed at 580°C, cooled and weighed accurately to obtain the ash weight. Duplicate samples of the ashed bone were prepared for phosphorus and calcium analysis using the procedure described in *Section 3.6.3.2.*

3.6.4.4 Measurement of phosphorus content

Preparation of phosphorus standards Aliquots of potassium phosphate ($K_2H_2PO_4$) standard solution containing 0.5, 0.8, 1.0 and 1.5 mg phosphorus were transferred to 100 ml volumetric flasks. 20ml molybdovanadate reagent was added, and the solution diluted to volume with distilled water, shaken thoroughly and allowed to stand for 10 -30 minutes. These standards were used in the determination of faeces, ileal digesta and bone samples. For determination of phosphorus content of the urine a series of standards having a lower concentration (0.1, 0.2, 0.3, 0.4, 0.5 mg) of phosphorus was prepared.

Construction of the standard curve A Pye Unicam UV Spectrophotometer was used to measure colorimetrically the *per cent* transmission of each standard solution. The wavelength was set at 400 nm and the 0.5 mg standard injected into the cell. The spectrophotometer reading was adjusted to 100, the cell was emptied and the 0.8mg standard injected. The new reading, which was less than 100, was recorded and this was repeated for the 1.0 and the 1.5 standard. For the urine samples, the most dilute standard of 0.1mg was set at 100%T and subsequent readings taken of the more concentrated standards (0.2-0.5mg). For each set of standards a simple linear regression was generated and the equation of the line used to predict the amount of phosphorus in the sample aliquot.

Estimation of phosphorus content of the samples After preparing the samples as in *Sections 3.6.3.1* and *3.6.3.2*, an aliquot of each solution was transferred to a 100ml volumetric flask and 20ml of molybdovanadate solution was added. The volume of the aliquot varied from 0.1-3.0 ml, depending on the nature of the sample. Bone analysis required only 0.2ml aliquots to bring the resulting transmission within the range of the standards, whereas urine required up to 5ml in order to obtain a detectable transmission. The size of the aliquot taken was, however, kept constant between like samples. The solution was made up to volume with distilled water, shaken thoroughly, and left to stand for 10-30 minutes.

The resulting solutions were injected into the spectrophotometer and the sample reading recorded. Each duplicate sample was injected twice into the spectrophotometer or until a constant reading was obtained. The reading obtained was used to calculate the amount of phosphorus (mg) in the corresponding sample using the equation of the line generated from the standard curve. This in turn was used to calculate the amount of phosphorus in the original sample using the equation :

% Phosphorus = mg phosphorus in aliquot/ (g sample in aliquot x 10)

3.6.4.5 Calcium analysis

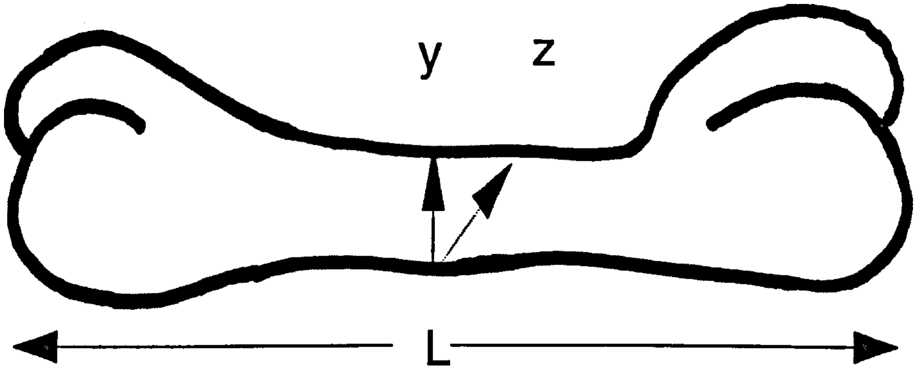
Prior to addition of ammonium molybdovanadate to the samples, a 10ml aliquot was collected for calcium analysis using atomic absorption (Varian SpectraAA 20) at a wavelength of 422.7nm. The standard range was 4-40ppm; some samples had to be diluted to 1 in 100 in order to achieve a reading between this range.

3.6.5 Determination of bone breaking strength

3.6.5.1 Preparation of bones for breaking strength determination

Bones were defrosted to room temperature before breaking tests. Extraneous tissue was removed with a blunt scalpel, taking care not to scratch the surface of the bone.

Figure 3.5 **Bone measurements taken during *Experiments 3* and *4***



During the dissection, the bones were kept moist in a solution of 0.9 molar saline. The third and fourth metatarsals of the piglets from the first growth trial were dissected apart in the laboratory; the metatarsals from the larger pigs of the second trial were separated at the slaughter house, following their removal from the carcase.

Bone length (l), the diameter across the midshaft of the bone (y) and the diameter perpendicular to y (z) were measured using vernier callipers (figure 3.5).

3.6.5.2 Flexure test

An Instron Universal testing machine (model 1140) was used to determine the maximum force that could be withstood by each bone. A force was applied at a constant speed of 100mm/min to the bone supported at each end, and a chart recorder set at 0.8mm/second was used to plot the force deformation curve. The test was stopped when the curve levelled off or reached a peak. This was considered to be the maximum force that could be withstood as although most bones did not break apart at this point, the bone failed to withstand any increases in force.

The femurs and the third and fourth metatarsals of the young pigs in the first growth trial (*Experiment 3*), and the third and fourth metatarsals of the growing pigs in the second growth trial (*Experiment 4*) were subjected to the flexure test.

3.6.6 Preparation of bones for mineral analysis

After mechanical testing, bones were autoclaved at 116°C at 1.3 kg/cm^2 for 10 minutes, and all remaining extraneous tissue was removed using a scalpel. Fat extraction of the third and fourth metatarsals was carried out using ether extraction (AOAC, 1980). This was impractical for the femurs due to their large size; these bones were therefore 'snap-frozen' in liquid nitrogen for 60 seconds, and crushed in a purpose-built hydraulic apparatus. The whole crushed bone was then agitated for 24 hours with a petroleum ether to remove fat. The whole femur, and the whole third and fourth metatarsals were ashed for 5 hours at 580°C and used for phosphorus and calcium determination as described in *Sections 3.6.3* and *3.6.4.*

3.6.7 Phytase activity

Phytase activity was measured by monitoring the release of inorganic phosphate from phytate using a Spectrophotometer at 335nm, as described Simons *et al.*, (1990). 1 unit was defined as the amount of enzyme which liberated 1μmol of phosphate from phytate in 1 minute at 37°C and pH 5.5.

3.7 Calculation of results

3.7.1 Calculation of digestibility results

Apparent total tract digestibility in the digestibility studies, apparent digestibility of nutrients as opposed to true digestibility was measured, as endogenous losses were not sequestered from faecal and urinary losses. Apparent total tract digestibility of each nutrient was calculated as a coefficient using the formula:

$$\text{Digestibility} = ((FI \times N_f) - (FO \times N_{fc}))/\ (FI \times N_f)$$

where FI = total feed intake during collection period

FO = total faecal output during collection period

N_f = nutrient concentration of the feed

N_{fc} = nutrient concentration of the faeces

Apparent ileal digestibility apparent ileal digestibility coefficients were calculated using the formula:

Digestibility $= 1 - ((N_d \times M_f) / (M_d \times N_f))$

where N_d = nutrient concentration in the digesta

N_f = nutrient concentration in the feed

M_d = marker concentration in the digesta

M_f = marker concentration in the feed

3.7.2 Calculation of mineral balance

The amounts of phosphorus and calcium retained were determined by subtracting urinary and faecal losses from the respective intakes during the collection period. These were expressed as absolute amounts (g), percentage of intake (I%), and percentage of that digested (Dg%).

3.7.3 Calculation of growth results

Mean daily feed intake from the recorded daily feed intake, the total feed consumed during the time on test was calculated and divided by the number of days on test to give the mean daily feed intake

Average daily liveweight gain was obtained from the slope of the linear regression of liveweight against 'days'

Feed conversion efficiency was calculated by dividing the total feed eaten during the experimental period by the total liveweight gain over the same period.

3.8 Statistical analysis

3.8.1 Pig metabolism trials

A latin square design was used for the two metabolism trials. The first experiment consisted of a replicated 3 x 3 latin square, having 3 treatments and 3 periods; the second used a triplicated 4 x 4 design with 4 treatments and 4 periods. Digestibility and balance data were subjected to analysis of variance using Genstat 5 (Lawes Agricultural Trust, 1984) to test for the significance of differences between the treatment means. Effects of treatment, period and pig were tested, and a blocked structure used to test for interactions between pig and period.

In the second metabolism trial, where the optimum level of phytase inclusion was being investigated, apparent phosphorus digestibility and retention were regressed against enzyme level (units/kg feed). Linear ($y = a + bx$) and curvilinear ($y= a + bx + cx^2$) models were produced by genstat 5, and a probability value denoting the goodness of fit of each line was generated.

3.8.2 Growth trials

For the growth experiments, analysis of variance was carried out to test effects of phosphorus level, phytase sex and the interactions on growth performance parameters. For the bone strength, bone mineral and carcase mineral data, analysis of covariance using 'slaughterweight' and 'days on test' as covariates was performed. Use of covariates was in order to dispense with the differences due to different finishing weights of the pigs, and due to the fact that some were more mature than others although the finish weight may have been equal. For the analysis of carcase measurements, finish weight was used as the sole covariate, as the amount of time spent on test by the animals was confounded with the differences in feed intake as a result of diet differences, and was anticipated to have a direct effect on measurements of fat and lean taken from the carcase.

Chapter 4 Experiment 1 - Effect of phytase and yeast on apparent digestibility of phosphorus in growing pigs

4.1 Introduction

In order to provide a basis for further research it was considered necessary to conduct a pilot trial to test the efficacy of dietary agents that reportedly improve phosphorus availability. Enzymic hydrolysis of phytates has been shown to increase the apparent digestibility of phosphorus and other nutrients associated with phytates, such as calcium and proteins. There may also be a beneficial effect of dietary yeast on phosphorus digestibility. Fermentation has formerly been associated with the hind-gut, but recent work (Dierick, 1989) indicates that some fermentation may occur anterior to this site. By using yeast to increase fermentation there may be an opportunity to liberate phosphorus from phytate before it reaches its site of absorption in the small intestine, and thereby improve its availability in the digestive tract. Furthermore, alteration of fermentation patterns in the large intestine and concomitant release of bound phosphorus from the soluble fibre with which it is associated, could promote any absorption of phosphorus that may take place beyond the ileum at this site.

In the experiment reported here, a high-phytate diet was used to study the effects of phytase on the dietary availability of phosphorus and other nutrients associated with phytates, and to determine whether an alteration in fermentation induced by dietary yeast influenced availability of phosphorus in the gut, using apparent digestibility as an indicator of dietary availability. Determination of apparent ileal and total tract digestibility of nutrients was made possible by using surgically modified animals fitted with a simple T-piece cannula. It was hoped to achieve an insight into the action of the dietary agents, and to the site of absorption of the nutrients, particularly phosphorus.

4.2 Materials and Method

4.2.1 Experimental design

The experiment utilised a replicated 3 x 3 latin square, consisting of 6 pigs tested over three periods. The treatments were:

C	control; unsupplemented basal diet
P	basal diet + 2.5g phytase/kg diet
Y	basal diet + 5g yeast/kg diet

The design of the latin square is given in figure 4.1

4.2.2 Animals

Six gilts weighing approximately 25 kg liveweight were used. A simple T-piece cannula was inserted into the terminal ileum of each animal under standard operating procedures, as described in *Section 3.5.1.2* of the materials and methods.

4.2.3 Diet

The basal diet was barley based and was formulated to contain a relatively low level of phosphorus (4.67 g/kg) with approximately half (2.34g/kg) present in the form of phytate phosphorus. The composition and nutrient specification of the basal diet are given in tables 4.1 and 4.2. Crude fibre content of the diet was relatively high in order to maximize response to the yeast.

Phytase produced by *Aspergillus niger* (var *ficuum*) having an activity of 400 units/g was added to the feed at a level of 2.5g/kg freshweight, providing a resultant activity of 1000 units/ kg feed.

Figure 4.1 Latin square design of experiment

	Period				Period		
Pig	I	II	III	Pig	I	II	III
1	y	c	p	6	y	c	p
2	c	p	y	3	c	p	y
4	p	y	c	5	p	y	c

Table 4.1 Formulation and phosphorus/phytate content of experimental diet

Ingredient	g/kg	phosphorus (g/kg FW)	total phosphorus contribution to diet	phytate phosphorus (%)	phytate-phosphorus contribution to diet
Barley	615	3.9	2.34	64	1.50
Soya 50	175	6.8	1.16	62	0.71
Rapeseed meal	60	6.7	0.37	35	0.13
Fat 50% premix	100	-	-	-	-
Soya oil	25	-	-	-	-
Betamix 314	12.5	-	-	-	-
Fish 66	10	44	0.44	-	-
Dicalcium phosphate	2	180	0.27	-	-
Synthetic lysine	0.5	-	-	-	-
Total	1000		4.67		2.34

Table 4.2 Nutrient specification of experimental diet

Nutrient	g/kg freshweight
Digestible energy (MJ/kg)	14.14
Crude protein	185.58
Oil	68.79
Crude fibre	49.19
Lysine	10.26
Methionine	3.12
Methionine + cystine	6.47
Threonine	6.74
Isoleucine	7.83
Tryptophan	2.45
Leucine	13.14
Histidine	4.33
Phenylalanine + tyrosine	15.01
Valine	9.22
Phenylalanine	4.01
Ash	53.08
Calcium	7.05
Phosphorus	4.67
Salt	3.63
Linoleic acid	13.44

The yeast used was a strain of *Saccharomyces cerevisae* (NCYC^{1026}), which contained approximately 5 x 10^9 organisms/g. This was added to the basal diet at a level of 5g per kg feed.

Powdered titanium dioxide (TiO_2) was used as a marker for the ileal digesta. This was added to the feed at a rate of 1g/kg freshweight.

4.2.4 Procedure

Following an initial post-operative recovery period, the treatment levels of feed were introduced and maintained for a 10-day acclimatization period, and thereafter throughout the first collection period of 10 days as described in *Section 3.5.2* of the preceding chapter. Total faeces and urine were collected for 5 days, followed by a 5-day ileal sampling period during which digesta was collected through the cannula four times daily on alternate days. This procedure was repeated for periods 2 and 3.

4.2.5 Statistical analysis

Digestibility data were subjected to analysis of variance (Genstat 5) to test for the significance of the difference between the treatment means, and to test for the significance of the difference between ileal and total tract digestibility.

4.3 Results

4.3.1 Health of the animals

Problems of cannula leakage occurred, particularly towards the end of the trial as increasing levels of feed imposed a stress on the gut around the site of the cannula. A full health report is given in Appendix II. Cannula leakage resulted in the loss of 2 of the animals before the end of the trial. In these cases, the mean digestibility values were based on the surviving animal for the treatment.

4.3.2 Analysis of the diet

Results from the laboratory analysis of the diet are shown in table 4.3. The actual phosphorus level was higher than that predicted during ration formulation (5.67 g/kg DM as opposed to 5.25 g/kg), which meant that the level of deficiency aimed for at the start of the trial was not wholly achieved.

4.3.3 Analysis of phytase and yeast

Mean phytase activity as measured under the standard assay procedure was 415 units/g (range 404-445 units; n=8). The phosphorus and nitrogen content of the phytase and yeast are given in table 4.4. These levels did not effect the overall dietary levels of phosphorus and nitrogen in the experimental diets.

Table 4.3 Laboratory analysis of experimental diet

Nutrient	g/kg dry matter
Dry matter (%)	89.0
Gross energy (MJ/kg)	19.98
Fat	79.28
Protein (N x 6.25)	184.42
Crude fibre	50.56
Phosphorus	5.67
Calcium	7.33
Ash	58.26

Table 4.4 Nitrogen and phosphorus content of phytase and yeast

	yeast(n=5)	phytase (n=5)
Nitrogen	23.4%	33%
Phosphorus	0.13%	0.24%

4.3.4 Phosphorus digestibility

A summary of the apparent ileal and total tract digestibility values is presented in table 4.5. Addition of phytase resulted in an increased ileal digestibility of phosphorus from 0.259 to 0.387 (p=0.094). This could be ascribed to hydrolysis of phytates occurring anterior to the ileum. Total tract digestibility was increased from 0.483 to 0.632 (p=0.074); a larger difference than was observed at the ileum (figure 4.2), suggesting that phytate hydrolysis was occurring further along the digestive tract. In each treatment case, relatively large differences in ileal and total tract values suggested the occurrence of post-ileal phosphorus absorption. There was no notable effect of yeast on either ileal or total tract phosphorus digestibility.

Values of phosphorus intake, faecal output, and digestibility obtained for each animal are given in Table 4.6. Pigs receiving phytase had a significantly lower phosphorus concentration in the faeces (1.121%, vs 1.627 control and 1.622 yeast; p = 0.05, sed = 0.171), which was reflected in the total faecal phosphorus excretion which, during the 5 day collection period was considerably lower for pigs fed the phytase diets (16.67g, vs 26.41g control and 23.72g yeast; p = 0.018, sed = 2.247).

4.3.5 Urinary phosphorus excretion

Urinary phosphorus excretion was low, varying from less than 30mg/litre up to 500 mg/litre, resulting in a 5-day output that ranged from less than 0.15g up to 4.85g. The urinary phosphorus concentrations across all periods for the four pigs that completed the trial are shown in table 4.7. Statistical analyses were not appropriate as in some cases urinary concentrations were so low as to make an accurate determination of the phosphorus output infeasible. However, it could be seen that for each animal, phytase addition resulted in an increased output of urinary phosphorus.

Table 4.5 Ileal and total tract digestibility of nutrients

	Treatment means			p	sed
	Control	*Phytase*	*Yeast*		
Ileal					
Phosphorus	0.259	0.387	0.283	0.094	0.054
Nitrogen	0.553	0.681	0.699	0.077	0.064
Calcium	0.479	0.492	0.483	0.985	0.078
Total Tract					
Phosphorus	0.483	0.632	0.478	0.074	0.070
Nitrogen	0.814	0.855	0.840	0.076	0.017
Calcium	0.660	0.652	0.652	0.677	0.049
Dry matter	0.784	0.794	0.797	0.745	0.016
Gross energy	0.772	0.784	0.773	0.768	0.017
Ether extract	0.706	0.736	0.771	0.273	0.039

Figure 4.2 Effect of phytase and yeast on apparent ileal and total tract phosphorus digestibility

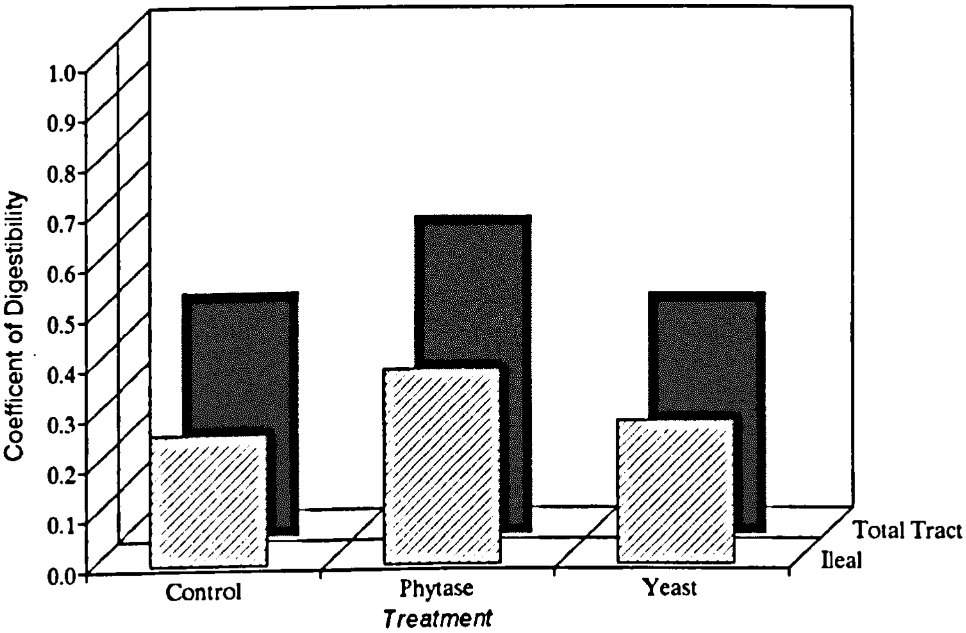


Table 4.6 Phosphorus balance of experimental animals

Pig no.	Treatment	P intake (g)	% P in faeces	P excreted in faeces (g)	Total tract digestibility	%P in digesta	Ileal digestibility
Period I							
1	y	46.006	1.330	19.010	0.587	0.860	0.272
2	c	46.006	1.325	19.328	0.580	0.689	0.271
3	c	36.484	1.350	14.476	0.603	0.800	0.203
4	p	34.363	1.144	9.402	0.725	1.193	0.423
5	p	-	-	-	-	-	-
6	y	22.998	1.313	9.288	0.598	0.885	0.296
Period II							
1	c	49.568	2.280	35.536	0.283	0.820	0.232
2	p	53.130	0.769	10.872	0.795	0.695	0.643
3	p	53.130	1.220	20.430	0.616	0.320	0.583
4	y	41.406	1.800	24.990	0.397	1.150	0.381
5	y	-	-	-	-	-	-
6	c	49.568	1.200	19.407	0.615	0.290	0.550
Period III							
1	p	58.769	1.590	34.426	0.414	1.170	0.127
2	y	-	-	-	-	-	-
3	y	62.331	2.040	34.536	0.446	1.060	0.184
4	c	56.362	1.780	34.507	0.398	1.080	0.150
5	c	-	-	-	-	-	-
6	p	37.992	1.113	17.291	0.552	1.450	0.123

Table 4.7 Urinary phosphorus excretion of experimental animals

Pig	Treatment	mg P/litre	5-day output (g)
Period I			
1	y	280	3.254
2	c	< 30	<0.414
3	c	< 30	< 0.494
4	p	160	0.801
5	p	-	-
6	y	< 30	<0.150
Period II			
1	c	170	0.272
2	p	60	0.964
3	p	60	0.678
4	y	60	0.534
5	y	-	-
6	c	130	1.369
Period III			
1	p	210	2.636
2	y	-	-
3	y	60	0.688
4	c	60	0.688
5	c	-	-
6	p	500	4.489

4.3.6 Calcium digestibility

Effects of phytase and yeast on apparent calcium digestibility are presented in table 4.8. There were no discernable effects of either phytase or yeast on ileal or total tract calcium digestibility ($P > 0.10$). Total tract digestibility of calcium was significantly higher than ileal digestibility ($p < 0.001$).

4.3.7 Nitrogen digestibility

Nitrogen digestibility coefficients are presented in table 4.9. Addition of phytase resulted in an increased ileal nitrogen digestibility from 0.553 to 0.681 ($p = 0.077$) and an increased total tract nitrogen digestibility from 0.814 to 0.855 ($p = 0.076$; figure 4.3). Dietary yeast inclusion caused an increased ileal nitrogen digestibility from 0.553 to 0.699, possibly due to stimulation of proteolytic bacteria since yeast itself is not actively proteolytic. This increase was not reflected in the total tract digestibility value, which, although higher than the ileal digestibility (0.84 vs 0.699) was not significantly higher than that obtained from pigs fed the control diet (0.814).

4.3.8 Digestibility of dry matter, gross energy, and ether extract

Increases in digestibility of dry matter, gross energy and fat with yeast and phytase addition were small and non-significant. results are summarized in table 4.10. It was not possible to measure the gross energy and fat content (ether extract) of ileal digesta due to small amount of sample remaining from other laboratory analyses. Furthermore, determination of dry matter digestibility at the site of the ileum was inappropriate due to the extreme variations in the nature of the digesta which ranged from semi-solid to liquid.

Table 4.8 Calcium digestibility

Pig no.	Treatment	Ca intake (g)	% Ca in faeces	Ca excreted in faeces (g)	Total tract digestibility	% Ca in digesta	Ileal digestibility
Period I							
1	y	38.833	0.928	13.26	0.659	0.614	0.455
2	c	38.833	.920	13.42	0.654	0.558	0.381
3	c	30.796	.934	10.015	0.675	0.402	0.580
4	p	29.007	1.012	8.347	0.712	1.270	0.356
5	p	-	-	-	-	-	-
6	y	19.417	0.892	6.29	0.676	0.690	0.563
Period II							
1	c	41.839	0.850	13.248	0.683	0.584	0.352
2	p	44.846	0.478	5.069	0.887	0.354	0.810
3	p	44.846	0.654	16.443	0.633	0.982	0.377
4	y	34.950	0.928	12.881	0.632	0.736	0.585
5	y	-	-	-	-	-	-
6	c	41.839	0.776	13.227	0.684	0.818	0.481
Period III							
1	p	49.606	0.990	21.434	0.568	0.654	0.488
2	y	-	-	-	-	-	-
3	y	52.612	0.754	12.765	0.757	0.726	0.355
4	c	47.602	0.764	17.467	0.633	0.674	0.540
5	c	-	-	-	-	-	-
6	p	33.555	0.901	11.691	0.652	0.654	0.567

Table 4.9 Ileal and total tract digestibility of nitrogen

Pig no.	Treatment	Ileal	Total tract
Period I			
1	y	57.50	79.77
2	c	47.48	76.71
3	c	35.83	79.10
4	p	73.78	84.80
5	p	-	-
6	y	71.95	81.61
Period II			
1	c	50.98	82.49
2	p	77.37	86.35
3	p	45.42	86.78
4	y	78.07	82.03
5	y	-	-
6	c	68	82.73
Period III			
1	p	62.35	84.72
2	y	-	-
3	y	66.77	89.10
4	c	64.64	83.60
5	c	-	-
6	p	75.90	85.59

Figure 4.3 **Effect of phytase and yeast on apparent ileal and total tract nitrogen digestibility**

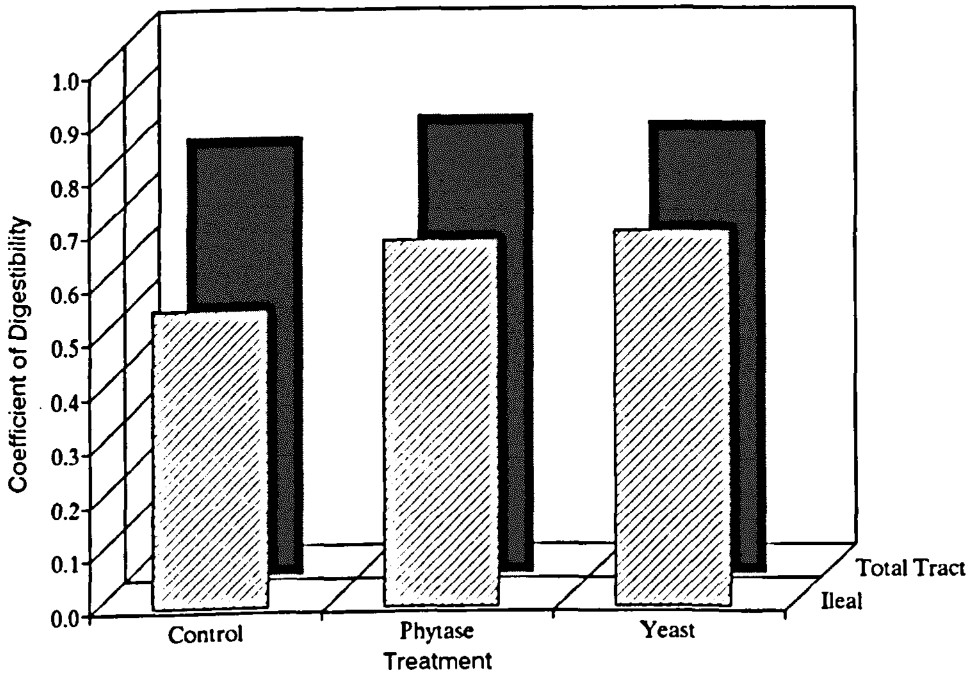


Table 4.10 Total tract digestibility of dry matter, gross energy and fat (ether extract)

Pig no.	Treatment	Dry matter	Gross energy	Ether extract
Period I				
1	y	0.793	0.778	0.715
2	c	0.789	0.775	0.712
3	c	0.804	0.761	0.723
4	p	0.840	0.834	0.824
5	p	-	-	-
6	y	0.796	0.775	0.955
Period II				
1	c	0.790	0.775	0.720
2	p	0.823	0.808	0.713
3	p	0.790	0.768	0.689
4	y	0.776	0.752	0.726
5	y	-	-	-
6	c	0.782	0.766	0.671
Period III				
1	p	0.754	0.743	0.694
2	y	-	-	-
3	y	0.819	0.791	0.752
4	c	0.771	0.778	0.704
5	c	-	-	-
6	p	0.731	0.717	0.675

4.3.9 Growth performance

Liveweight changes of the individual animals during each period are presented in table 4.11. Due to the surgical modification of the animals, and their periods of confinement in metabolism crates, growth performance was substantially lower than would be expected in a commercial environment.

4.4 Discussion

4.4.1 Cannulation of experimental animals

Cannulation of the pigs enabled a comparison of fore and hind-gut digestion and gave insight into the sites of action of the dietary treatments. Cannulation of the small intestine allows many collections of digesta from the same animal and avoids the effects of slaughter on epithelial cell loss. In the duodenum, the liquid phase flows much faster than the solid phase, a phenomenon associated with gastric emptying (Low *et al.*, 1978). It was therefore necessary to obtain samples at regular intervals after feeding in order to achieve steady state conditions. In this trial, sedimentation due to the fibrous nature of the diet caused difficulties in obtaining a representative sample of digesta.

Food intake, growth, and feed conversion efficiency have been shown to be reduced by the insertion of simple T-piece cannulae. (3%, 6% and 4% respectively; Livingstone, 1982). Cannulation could affect the microbial ecology of the gut, the anaerobic conditions at the site of the cannula and perhaps even the digestive processes. Unavoidable leakage of digesta around the site of the cannula exacerbates these changes. Alternative methods for the collection of ileal digesta include the re-entrant cannula, the ileo-colic post valvular procedure, and the ileo-rectal anastomosis technique; these have been reviewed by Jagger (1987).

Table 4.11 Liveweight changes during metabolism experiment

Liveweight (kg)

Pig	Period I		Period II		Period III	
	Adaptation	*Collection*	*Adaptation*	*Collection*	*Adaptation*	*Collection*
1	32.5	38	45	50	65	73
2	33	38	47	57	-	-
3	32	39	45.5	52.5	62.5	72
4	24	27.5	37	44	53	63
5	30	33	-	-	-	-
6	32	38	40	50	59	68

The nature of this experiment made it impractical to use more than 3 treatments as cannula leakage was noticeable by the end of the third collection period due to increased weight of digesta. Although it has been suggested that an acclimatization period of 10 days may not be enough for dietary adaptation, a longer period would have meant that effects of age on phosphorus requirement may have become apparent. The animals were not being adapted to a wholly new diet as the basal ingredients remained constant, and in this case a 10 day adaptation was probably sufficient.

4.4.2 Use of apparent digestibility

Apparent digestibility is not generally considered as being an accurate indicator of phosphorus availability. However, in this experiment a low phosphorus, high phytate diet was used in order to minimise endogenous losses. Indeed, it can be assumed that only the obligatory amount of endogenous phosphorus was lost, which consisted of the phosphorus secreted with gastric, pancreatic, bile and intestinal juices. Obligatory endogenous losses in the faeces has been estimated at 10mg/kg/day under normal feeding conditions (Guéguen and Perez, 1979). The overall importance of these losses to gastro-intestinal phosphorus turnover was therefore minor. Thus, because phosphorus absorption across the gut wall was not regulated under the conditions of a low phosphorus level, apparent digestibility could be used as a realistic indicator of dietary phosphorus availability.

4.4.3 Diet

The purpose of using a high phytate diet was to maximize response to the phytase, similarly, a high relatively level of crude fibre was used to maximize response to the yeast. Use of a low phosphorus diet was in order to reduce endogenous losses to those which were obligatory. It could be assumed that approximately one half of the dietary phosphorus in the control diet was able to be utilized. This was confirmed by total tract digestibility results of pigs on the control diet; mean phosphorus digestibility was 0.48 which corresponded to 2.30 g/kg of digested phosphorus (compared with 2.34 g/kg non-phytate phosphorus which was estimated to be the amount of digestible phosphorus).

Diets were fed in meal form because effects of pelleting on phosphorus digestibility were uncertain. Mechanical disruption of the cell walls of cereals releases intrinsic phytase from the globoid bodies into contact with phytate (Pointillart, 1993) and this may have confounded the digestibility results.

4.4.4 Experimental design

The latin square design allowed a relatively large number of replicated observations from few animals. The experiment used six gilts in a duplicated 3 x 3 latin square, from which 18 replications were achieved. Few animals were used because of the complexity of cannulation. However, this meant that sensitivity of the comparison of means was reduced because the main effects of treatments were compared to the mean square for animals within the treatment, which could be inflated by positive correlations among repeated observations (Gill, 1986). A coefficient of variation in digestibility of around 8-10% was expected, based on results of a previous trial (Cole *et al.*, unpublished data), thus, in order to detect a 10% difference in digestibility with 80% certainty, a minimum of 12 replicates were required.

Actual coefficients of variation encountered in digestibility results deviated widely from the expected 8-10%; variation in ileal digestibility being generally much larger than between total tract digestibility. For example, coefficient of variation for ileal phosphorus digestibility was 52.5%, whereas for total tract phosphorus digestibility was 9.1%. It is accepted that large variation in phosphorus metabolism exists between animals, and present results indicated that this variation should be given due consideration when formulating phosphorus allowances.

4.4.5 Phosphorus balance

When phytase was added to the basal diet a 50% improvement in apparent ileal digestibility of phosphorus was obtained, from 0.26 to 0.39. In a similar cannulation experiment (Simons *et al.*, 1990) an increased ileal phosphorus digestibility from 0.20 to 0.46 was observed, and it was concluded that approximately 50% of the phytic phosphorus present in a maize/soybean diet was made available by use of the enzyme.

Results were similar to those of Jongbloed *et al.* (1992) who also used 6 pigs fed diets based either on corn-soybean or tapioca-hominy feed. Addition of phytase to the former increased ileal digestibility from 0.26 to 0.45, and with the tapioca-hominy diet an increase from 0.16 to 0.46 was observed. Total tract digestibility increased from 0.13 to 0.43 (maize-soya) and from 0.27 to 0.55 (tapioca-hominy).

Supplementation of yeast into the control diet gave no notable improvements in either ileal or total tract phosphorus digestibility. It has been suggested that the phytase produced by *Saccharomyces* may result in increased phosphorus availability but our results did not indicate this. On the other hand, the expected increase in digestibility would be less than that achieved with phytase, suggesting that greater replication may have been needed.

The reduction in faecal phosphorus concentration obtained with phytase resulted in a 37% lowering of total faecal phosphorus output, and was similar to the reduction of 37% obtained by Simons *et al.* (1990) using pigs of a similar liveweight. A strong period effect ($p=0.001$) on the amount of phosphorus excreted was noted. Although the faecal phosphorus concentration remained fairly constant, the increasing amount of faeces excreted as the pigs grew resulted in an increased excretion of phosphorus. This reflected directly the amount of phosphorus taken in, rather than changes in digestibility, and indicated that the concentration of phosphorus in the feed should be decreased in several phases towards the finishing stage.

The low urinary phosphorus concentrations obtained when pigs were fed the control diet indicated that the animals were deficient in phosphorus. The level of digestible phosphorus, at 2.34/kg, was lower than the NRC (1988) and the ARC (1981) recommendations for digestible phosphorus, but corresponded to requirements proposed (Jongbloed *et al.*, 1991) for pigs of 30-50kg liveweight (2.3-2.0g/kg). Present results suggested that the latter estimates of requirements, which were obtained using factorial estimates, would not satisfy the requirements of growing pigs.

When phytase was added to the diet the increased dietary availability of phosphorus resulted in a greater absorption into the body pool. In order to counteract the increase, some of the absorbed phosphorus was off-loaded into the urine. On the whole, urinary phosphorus excretion was fairly inconsistent and followed no clear trend. For example, the two pigs that started the trial on the control diet appeared to have been extremely deficient in phosphorus as addition of phytase did not greatly increase their phosphorus excretion. The skeletal system acts as a buffer against low blood phosphorus during dietary deficiency of phosphorus. It could be presumed that upon addition of phytase, some dietary phosphorus was used to make up the skeletal deficit that had been induced in these two pigs which were receiving the control diet for an extended period (ie during the recovery period until the end of the first experimental period). This was reflected in a maintained low urinary phosphate excretion.

The fact that in only one of the yeast-fed animals was there an increased urinary phosphorus excretion (over the control) suggested that the animals receiving yeast suffered a similar phosphorus deficiency as the control animals. Results were consistent with the digestibility results, ie little or no extra phosphorus was made available by yeast, and therefore no excess phosphorus was excreted into the urine.

4.4.6 Calcium digestibility

The lack of increase in calcium digestibility due to microbial phytase was surprising as calcium ions are bound within phytates and were expected to have been released upon degradation of the phytate complex. Other workers (eg Simons *et al.*, 1990;

Lantzsch and Wjst, 1992; Hoppe *et al.*, 1992) reported increased calcium digestibility when microbial phytase was supplemented to the diet. Mroz *et al.* (1993) found that the effect of phytase on calcium was dependent on the dietary calcium level. These workers tested phytase with levels of 4,6 and 8g/kg calcium and found the maximum response at the lowest level of dietary calcium.

The barley based diet used meant that the phytate was present in the form of potassium and magnesium salts unlike the calcium-magnesium complexes present in most other cereals (Pomeranz, 1973). This may explain the lack of response of calcium. Furthermore, the diet was not designed to be deficient in this mineral. Absorption of calcium across the gut wall can be regulated to suit requirements. Therefore even if availability was improved, the fact that it was already sufficient in the diet may have prevented further absorption.

Feedstuffs rich in phytate also have a high concentration of fibre, and it has been suggested (Reinhold *et al.*, 1975) that fibre rather than phytate largely determines the availability of bivalent metals for absorption. However, due to the lack of response with either yeast or phytase it was not possible to judge whether fibre or phytate was the main determinant of calcium availability.

4.4.7 **Nitrogen digestibility**

The increased nitrogen digestibility obtained due to microbial phytase was similar to that observed by Officer and Batterham (1992). These workers found increases of between 7 and 12 percentage units in the ileal digestibility of crude protein and essential amino acids when phytase was used. Results indicated that the phytate protein complexes which build up in acid medium by amino groups or in alkaline medium by Ca^{2+} ions (Maga *et al.*, 1982) were being degraded during phytate hydrolysis. The effect may have been partly related to an increased proportion of liberated orthophosphates in the gut lumen changing the ratio of nitrogen to digestible phosphorus, resulting in an improved nitrogen absorption, as proposed by Mroz *et al.*

(1991). These workers noticed an increased ileal digestibility of methionine, cystine, arginine, isoleucine and phenylalanine with phytase. Total tract digestibility of all these amino acids, except lysine, was increased. In contrast, no effect of microbial phytase on total tract digestibility of protein was observed by Nasï (1991).

The increased ileal digestibility due to yeast inclusion may have been due to stimulation of proteolytic bacteria since yeast itself is not actively proteolytic. This increase was not reflected in the total tract digestibility, suggesting that perhaps the dietary yeast was acting on the proportion of protein that would normally be degraded in the hindgut, in which case it would be of more benefit. Reduced availability to hindgut micro-organisms could cause an alteration of hindgut fermentation patterns, but it was assumed that these changes did not create any improvement in phosphorus digestibility as if so, there would have been an improved total tract digestibility.

4.4.8 Energy

No notable difference in gross energy digestibility between the diets was observed. Differences may have been too small to detect with the number of replicates used. Supplementation of yeast into the diet would be expected to achieve an improved energy digestibility, particularly due to the fibrous nature of the diet. It has been suggested that phytate inhibits alpha-amylase activity (Knuckles and Betschart, 1987), thus there may be associated effects of phytase on starch digestibility. However, this was not apparent from these results.

4.4.9 Site of action of phytase and yeast

No measurable phytase activity was detected in the ileal digesta or the faeces, suggesting that the *Aspergillus niger* phytase was active anterior to the terminal ileum. Previous workers (eg Guéguen *et al.*, 1968, Jongbloed *et al.*, 1992) agreed that a substantial degradation of phytic acid occurs in the gastroduodenum. Lantzsch *et al.*, (1992) concluded that approximately 70% of total dietary phytic acid was hydrolysed by the time digesta had reached the end of the small intestine.

Physico-chemical properties of soluble and insoluble dietary fibres indicate that they affect digestion and absorption processes in different sections of the gut (Jongbloed and Kemme 1990). Enzymes which cleave the bonds of non-starch polysaccharides (NSP) are normally not present in the small intestine of pigs. Most NSP are recovered at the terminal ileum, although some are broken down by the microflora in the large intestine. From the increase in nitrogen digestibility obtained with dietary yeast it could be inferred that it influenced the digestive contents proximal to the ileum. Yeast functions by influencing the gut microbial populations rather than a direct effect upon specific nutrients. In horses, dietary yeasts survive through the small intestine to the caecum (Pagan 1989), and in ruminants, yeasts flow out of the rumen amongst the digesta (Chesson, 1992). Thus it could be postulated that the yeast began its effect in the stomach and continued thereafter throughout the digestive tract.

4.4.10 Absorption of phosphorus

A substantial increase in apparent ileal phosphorus digestibility suggested that absorption of phosphate was occurring anterior to the terminal ileum, but differences in ileal and total tract digestibility values also indicated the occurrence of post-ileal absorption. It was previously reported (Lantzsch *et al.*, 1992) that due to plant phytase, 55% of phytate-phosphorus from maize was absorbed by the end of the small intestine, and nearly 40% of the absorption took place in the proximal half. Their post-slaughter studies showed that plant and microbial phytases in the stomach and small intestine caused hydrolysis of approximately 33% of ingested phytate-phosphorus within 3 hours, while around 42% was released 9 hours post-prandially. However, Jongbloed *et al.* (1992) determined that although phytate complexes were hydrolysed in the stomach and duodenum, liberated ortho-phosphates were not absorbed until they reached the ileum. Results of the present trial indicated that phosphorus was being absorbed further along the digestive tract. The hind-gut has previously been dismissed as insignificant for phosphorus absorption in pigs, but early work (Partridge, 1978) indicated some net absorption of phosphorus, dependent on the dietary level, and recently, Jongbloed *et al.* (1992) found a net disappearance of phosphorus distal to the terminal ileum.

4.4.11 Level of phytase and yeast used

The level of phytase used (1000 units/kg) had previously been shown to be efficacious in obtaining an increase in phosphorus digestibility (eg Simons *et al.*, 1990; Jongbloed *et al.*, 1992), and was sufficient in this experiment to achieve a substantial degradation of phytic phosphorus. The rate of dietary yeast inclusion was based on the amount of a similar culture used by Pagan (1989). Using 10g yeast/day, increases in phosphorus digestibility ($p<0.01$) were achieved. It may have been that the level of yeast used in our diets were not high enough to exert an effect. On the other hand, when 15 g/kg *Saccharomyces cerevisiae* were added to a maize soya diet for pigs, no improvement in the availability of phosphorus as measured by growth performance and bone strength was observed (Cromwell and Stahly, 1978)

4.4.12 Efficacy of phytase and yeast

The digestive tract of the pig offers limited opportunity for exogenous enzyme action due to the pH, the high viscosity of digesta, and the lack of discreet compartmentalisation of the digestive contents. Nonetheless, it seemed that the phytase from *Aspergillus* was sufficiently active *in vivo* to cause phytate hydrolysis and concomitant release of orthophosphates which were then absorbed. Degradation of phytate takes place in a stepwise manner, producing the intermediates IP_1, IP_2, IP_3, IP_4 and IP_5. Only IP_1, the free inositol phosphate, can be absorbed across the gut membrane. It has been previously indicated that due to the diversity of phytate products, it may be necessary to use different enzymes, each specific for a particular inositol phosphate. Intermediate compounds were not measured, however, based on the large increase in phosphorus digestibility obtained with the phytase, it was assumed that phytate compounds were degraded to free inositol phosphates. It was calculated (table 4.12) that approximately one third of the phytate-phosphorus present in the diet was made digestible in the small intestine by using phytase.

The phytase used was a crude, unpurified preparation which contained other enzymes such as cellulases, proteases and amylases. It may be argued that improved digestibility of other nutrients associated with phytates, such as proteins, was brought about by these exogenous enzymes rather than due to an effect of phytase. However, the activity of these associated enzymes was much lower than would be needed to show a notable response (table 4.13).

Aleurone secondary cell wall material contains cellulose, arabinoxylans and phytates. During digestion the cell wall breaks up giving access to enzymes. Hence an increased phosphorus digestibility with dietary yeast would have been expected. In view of the apparent post-ileal absorption of phosphorus it would be interesting to study the inclusion of both yeast and phytase into the diet to alter fermentation and phosphorus absorption simultaneously. It may be postulated that increased availability of substrate due to pre-caecal fermentation would precede hydrolysis of phytate, resulting in a further improvement of phosphorus digestibility.

4.5 Conclusions

It was concluded that the addition of 1000 units of *Aspergillus niger* phytase/kg diet was sufficient to cause phytate hydrolysis and therefore an increased phosphorus digestibility. Any alteration in fermentation as a result of yeast addition did not influence phosphorus digestibility. In practical diets fed to growing pigs, one half to two thirds of organic phosphorus is phytic, thus phytase could be used to in these diets to replace a large proportion of supplemented inorganic phosphate.

Table 4.12 Calculation of the amount of phosphorus made digestible due to phytase addition

Phosphorus content of diet = 4.67 g/kg
Phytate content of diet = 2.34 g/kg

Ileal phosphorus digestibility without phytase = 0.26
Digested phosphorus = 0.26 x 4.67
= 1.21g
Ileal phosphorus digestibility with phytase = 0.39
Digested phosphorus = 0.39 x 4.67
= 1.82g
Amount of phosphorus made digestible = 1.82 - 1.21
= 0.61 g
Assume all extra phosphorus digested is phytate
Proportion of phytate digestible due to phytase = (0.61/2.34) x 100%
= 26%

Table 4.13 Activity of enzymes other than phytase present in the enzyme preparation

Enzyme	units	activity (units/g)	relative significance[τ]
Cellulase	Filter paper units	18	insignificant
Alpha-amylase	Bacterial amylase units	0	insignificant
Protease	Haemoglobin units on tyrosine basis	725	insignificant

[τ] Comparison with cellulase, alpha amylase or protease enzyme preparation

Chapter 5 Experiment 2 - Determination of the optimum dietary phytase level

5.1 Introduction

The use of phytase to lower phosphorus excretion of pigs has been reported (eg Simons *et al.*, 1990, Beers and Jongbloed, 1992, Cromwell *et al.*, 1993, Ketaren *et al.*, 1993). However, information pertaining to dose-response relationships between phytase level and phosphorus digestibility is insufficient to enable determination of an optimum phytase level. Typically, 800-1000 units/kg feed is the defined dose but this may not represent the optimum. Young *et al.* (1993) obtained an increase in apparent phosphorus digestibility from 0.63 to 0.71 with the addition of 500 units phytase/kg but this increased only to 0.74 when the amount of enzyme was doubled. In contrast, plasma phosphate concentration of weanling pigs increased linearly in response to graded levels of phytase up to 750 units/kg (Lei *et al.*, 1993).

Differences in enzyme source and preparation between batches may be reflected as inconsistencies between results obtained from separate experiments, hence standardisation of assay conditions across experiments is important. One unit of phytase activity is defined as the amount of enzyme necessary to liberate 1 μmol of phosphate from phytate at 37°C and pH 5.5. Activity depends upon the source of the phytase-secreting microbe and the method of preparation. It is thus inappropriate to compare responses at different levels between experiments using different batches of the enzyme, unless the standard assay procedure has been followed.

In order to provide a basis for future trials, it was considered necessary to conduct a metabolism trial to investigate the relationship between level of phytase inclusion into the diet and phosphorus digestibility, so that the optimum level of dietary phytase inclusion could be determined.

5.2 Materials and Method

5.2.1 Experimental Design

12 gilts weighing approximately 25kg liveweight were randomly allocated to 1 of 4 treatments and used in a triplicated 4 x 4 latin square experiment. The treatments consisted of an unsupplemented basal diet (1), to which was added 500 phytase units/kg (2), 1000 units/kg (3) and 1500 units/kg (4). A plan of the latin squares is given in figure 5.1

5.2.2 Diet

The basal diet was a maize/soya based diet (table 5.1) which contained a low level of digestible phosphorus. Of the 5g/kg present in the diet approximately 2g were present as phytate phosphorus. Calcium:phosphorus ratio was 1.5:1. The nutrient specification of the experimental diet is presented in table 5.2

Activity of the phytase was approximately 400 units/gram. The enzyme was premixed into 10kg batches of the diet. The resulting diets A, B, C, and D contained 0, 1.25, 2.5g, and 3.75 grammes of phytase/kg respectively.

5.2.3 Procedure

The procedure followed was that described in *section 3.5.2* of the General Materials and Methods. In total, there were four periods, each consisting of a 10 day acclimatization followed by a 5-day total collection period. During the collection period, the animals were catheterised and restrained in metabolism crates.

Figure 5.1 Latin square design of experiment

	PERIOD			
PIG	I	II	III	IV
1	A	D	C	B
2	B	A	D	C
3	C	B	A	D
4	D	C	B	A

	PERIOD			
PIG	I	II	III	IV
5	A	D	C	B
6	B	A	D	C
7	C	B	A	D
8	D	C	B	A

	PERIOD			
PIG	I	II	III	IV
9	A	D	C	B
10	B	A	D	C
11	C	B	A	D
12	D	C	B	A

Table 5.1 Composition and phosphorus/ phytate content of experimental diet

Ingredient	g/kg	phosphorus (g/kg FW)	Total phosphorus contribution to diet	phytate-phosphorus (%)	phytate-phosphorus contribution to diet (g/kg)
Maize	450	2.6	1.17	66	0.78
Soya 50	180	6.8	1.22	61	0.75
Spring peas	150	4.3	0.64	50	0.32
Wheat	110	3.3	0.36	73	0.27
Fat 50% premix	90	2.0	0.18	-	-
Betamix 314	12.5	-	-	-	-
Dicalcium phosphate	8.0	180	1.44	-	-
Lysine	0.25	-	-	-	-
Threonine	0.08	-	-	-	-
Total	1000.8		5.01		2.12

Table 5.2 Nutrient specification of experimental diet

Nutrient	g/kg freshweight
Dry matter (%)	88.03
Digestible energy (MJ/kg)	14.89
Oil	68.14
Crude fibre	35.57
Lysine	10.02
Methionine	2.68
Methionine + cystine	5.72
Threonine	6.52
Calcium	7.33
Phosphorus	5.01
Ash	49.84
Linoleic acid	15.0
Salt	3.03

5.2.4 Laboratory Analysis

Samples of feed and faeces were analyzed for dry matter, gross energy, crude protein (N x 6.25), calcium and phosphorus as reported in *Section 3.6* of the General Materials and Methods. Phosphorus content of the urine was determined colorimetrically at 400nm using vanadium molybdate as the reducing agent.

5.2.5 Statistical Analysis

Digestibility and balance data were subjected to analysis of variance using Genstat 5 (Lawes Agricultural Trust) to test for the significance of the difference between the treatment means. Effects of phytase level, period and pig were tested, and a block structure used to test for a pig x period interaction.

Phosphorus digestibility was regressed against phytase level (units/kg diet). Linear and curvilinear models were produced by Genstat 5 and a probability value denoting the goodness of fit of each line was generated.

5.3 Results

5.3.1 Health of the animals

During the second collection period pig number 5 (basal diet +1500 units/kg) refused feed for 2 days, consequently the data from the animal for this period were excluded, and 'missing values' generated by Genstat 5 during the statistical analysis. No notable differences in growth rate occurred amongst the treatment groups; mean growth rate was 590 g/day (range 530-670 g/day).

5.3.2 Phosphorus balance

A summary of the results of the phosphorus balance is presented in table 5.3. A marked variation in phosphorus digestibility between animals was observed ($p=0.007$, $sed=0.043$). In addition, there was a decrease in digestibility from the first to the final period ($p=0.094$; table 5.4). Addition of phytase at 500, 1000 and 1500 units/kg to the basal diet resulted in an increased phosphorus digestibility ($P=0.054$), however, following the initial increase in digestibility when 500 units phytase/kg were added to the diet, any further changes in phosphorus digestibility with increased phytase addition were relatively small and non-significant. Corresponding to the increase in phosphorus digestibility with phytase, faecal phosphorus concentration decreased with addition of the enzyme, on average, from 1.6% without phytase, to 1.3% with phytase ($p=0.085$). Output of phosphorus increased significantly ($p<0.001$) from the first to the last period, while there was a tendency of a reduction in phosphorus digestibility from periods I to IV.

Urinary phosphorus excretion increased quite distinctly ($P<.001$) with increasing phytase additions (figures 5.2 and 5.3). Urinary phosphorus concentration gives an indication of the phosphorus status of an animal, as homeostasis occurs via the kidney. The animals receiving the basal diet which contained low amounts of digestible phosphorus were excreting only minimal amounts via the urine, but as the amount of digestible phosphorus effectively increased due to enzyme addition, increasing quantities of phosphorus were excreted by this route.

No significant differences in phosphorus retention between the treatments were found, furthermore there were no notable differences in phosphorus retention as a proportion of dietary phosphorus intake between the diets. Phosphorus retained as a proportion of that digested decreased from period I to IV (92.1% to 77.2%; $p=0.002$).
Regression analysis performed on the digestibility data showed that the response was best described as a quadratic curve ($p=0.048$; figure 5.4). The equation of the curve was $y = 0.583 + 0.00016x - 0.84E^{-7}x^2$ ($y=$ phosphorus digestibility, $x =$ units phytase/kg feed). From this it was seen that the maximum response was achieved using 900-1000 units phytase/kg.

Table 5.3 Phosphorus balance data

	Treatment means					
	Basal	+ 500	+ 1000	+1500	p	sed
Intake (g/day)	8.71	8.92	8.93	9.09	ns	1.374
Concentration in faeces (g/kg)	16.12	12.92	13.05	13.19	0.085	1.418
Total output in faeces (g)[τ]	17.60	16.50	16.50	16.79	ns	2.271
Digestibility	0.59	0.64	0.65	0.64	0.051	0.025
Concentration in urine (g/100g)	0.011	0.040	0.043	0.055	<0.001	0.008
Total output in urine (g)[τ]	1.92	5.42	6.36	7.92	<0.001	1.160
Retained (g/day)	4.80	4.53	4.49	4.38	ns	0.35
Retained / intake (%)	56.0	50.6	49.6	48	ns	4.26
Retained / digested (%)	92.1	80.0	77.0	77.2	0.002	4.15

[τ] In 5-day collection period

Table 5.4 Period effect on phosphorus balance

	Period Means					
	I	II	III	IV	p	sed
Intake (g/day)	7.95	7.26	9.26	9.38	<0.001	1.12
Concentration in faeces (g/kg)	1.38	1.24	1.45	1.48	ns	0.148
Total output in faeces (g)[r]	13.03	15.50	17.01	21.73	<0.001	1.824
Digestibility	0.66	0.63	0.64	0.59	0.094	0.025
Concentration in urine (g/kg)	0.035	0.051	0.025	0.001	ns	0.040
Total output in urine (g)[r]	4.45	6.29	5.48	5.40	ns	1.16
Retained (g/day)	4.80	4.53	4.49	4.38	ns	0.35
Retained / Intake (%)	56.0	50.6	49.6	48	ns	4.26
Retained / Digested (%)	92.1	80.0	77.0	77.2	0.002	4.15

[r] In 5-day collection period

Figure 5.2 Urinary phosphorus concentration

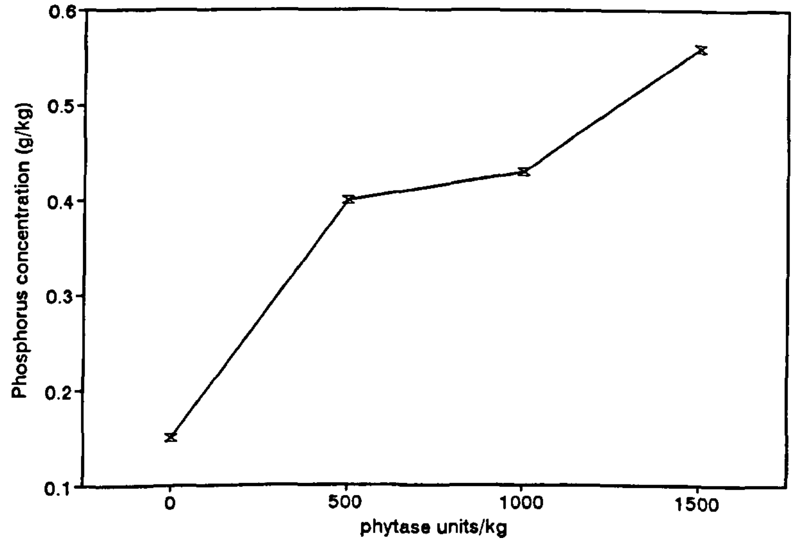


Figure 5.3 Total urinary phosphorus excreted during 5-day period

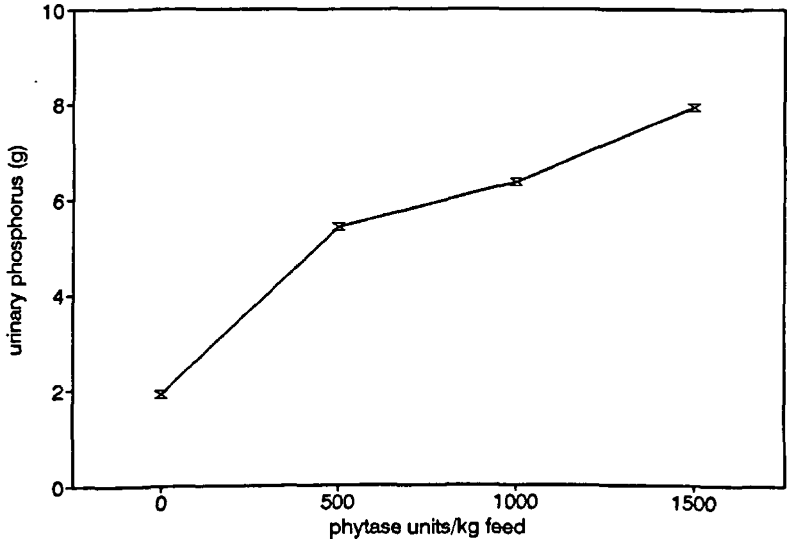
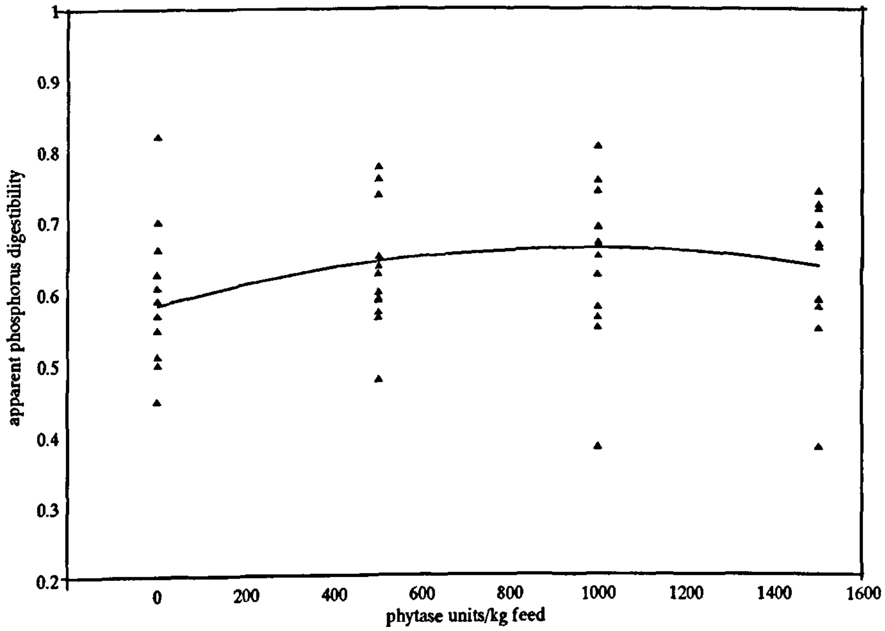


Figure 5.4 Relationship between phytase level and apparent phosphorus digestibility



$y = 0.583 + 0.00016x - 0.84E^{-7}x^2$ ($p=0.048$, % var = 9.1)

y = phosphorus digestibility

x = units phytase/kg feed

5.3.3 Calcium balance

Calcium balance results are summarised in tables 5.5 and 5.6. Mean digestibility decreased from the first to the last period (from 0.86 to 0.71, $p<0.001$). However, no notable changes in digestibility of calcium were achieved due to enzyme addition. Furthermore, the concentration of calcium in the faeces and the amount excreted during each period were unaltered by phytase addition. In contrast, the concentration and total amount of calcium in the urine were significantly lowered ($P=0.04$, $P=0.02$, respectively) with the addition of 500 units phytase/kg diet. This was reversed at the higher level of phytase inclusion, so that a greater concentration and subsequently a greater amount of urinary calcium was excreted (figures 5.5 and 5.6).

5.3.4 Ash digestibility

Mean ash digestibility coefficients are presented in table 5.7. Although the probability value obtained indicated that differences between treatments were non-significant ($p>0.10$), the difference in ash digestibility between the control and the highest level of phytase inclusion (from 0.49 to 0.58) suggested that there was an effect of the enzyme at the highest inclusion rate. There was a strong period effect ($p<0.001$) which probably reflected the changes in calcium digestibility.

5.3.5 Nitrogen, energy and dry matter digestibility

Mean digestibility coefficients for nitrogen, energy and dry matter are given in table 5.8. No influence of phytase on nitrogen or gross energy digestibility of the diets was detected. There was a slight decrease in dry matter digestibility between the basal diet and the 1000 units/kg diet, and an increase between the basal diet and the 1500 units/kg diet.

Table 5.5 Calcium balance data

	Treatment means					
	Basal	+ 500	+ 1000	+1500	p	sed
Intake (g/day)	12.0	13.4	13.8	13.0	ns	1.63
Digestibility	0.78	0.77	0.79	0.80	ns	0.22
Concentration in faeces (g/kg)	11.1	11.7	11.1	11.3	ns	0.59
Total faecal Ca output (g)[r]	13.2	15.4	14.5	13.0	ns	1.22
Concentration in urine (mg/ml)	860	360	620	1160	0.04	270
Total urinary Ca output (g)[r]	11.3	4.2	9.58	16.4	0.02	3.64
Retained Ca (g)[r]	30.5	39.0	34.4	35.1	ns	3.70
Retained / intake (%)	51.9	58.1	54.3	54.3	ns	3.97
Retained / digested (%)	58.8	74.5	66.6	67.4	ns	6.38

Table 5.6 Period effect on calcium balance

Period Means

	I	II	III	IV	p	sed
Intake (g/day)	12.1	10.2	13.4	15.1	<0.001	0.822
Concentration in faeces (g/kg)	8.7	9.19	11.43	15.82	<0.001	0.586
Total output in faeces (g)[r]	8.45	10.34	15.43	21.89	<0.001	1.220
Digestibility	0.86	0.82	0.77	0.71	<0.001	0.215
Concentration in urine (g/kg)	1.05	0.63	0.31	1.00	0.037	0.270
Total output in urine (g)[r]	11.8	6.9	7.6	15.1	ns	3.64
Retained (g/day)	7.16	6.8	6.48	7.38	ns	0.74
Retained / intake (%)	65.1	58.1	46.2	49.1	<0.001	3.97
Retained / digested (%)	69.7	71.0	57.1	69.4	ns	6.38

[r] In 5-day collection period

Figure 5.5 Urinary calcium concentration

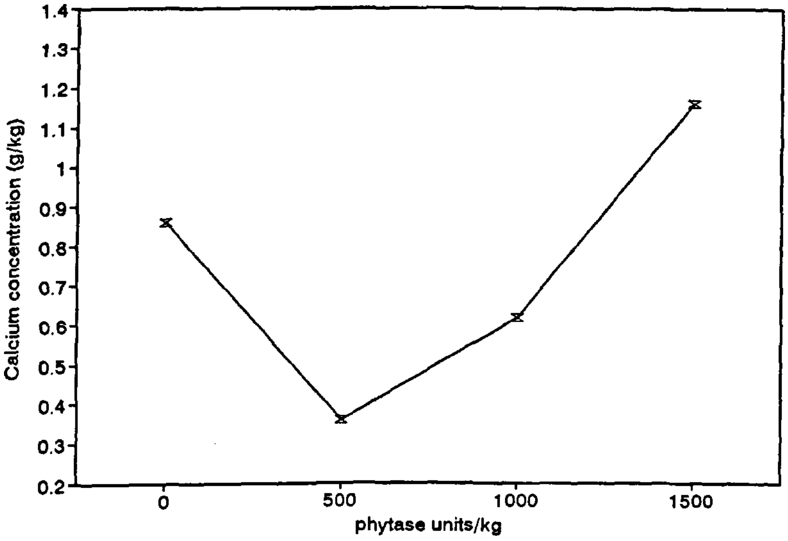


Figure 5.6 Total urinary calcium excreted during 5-day period

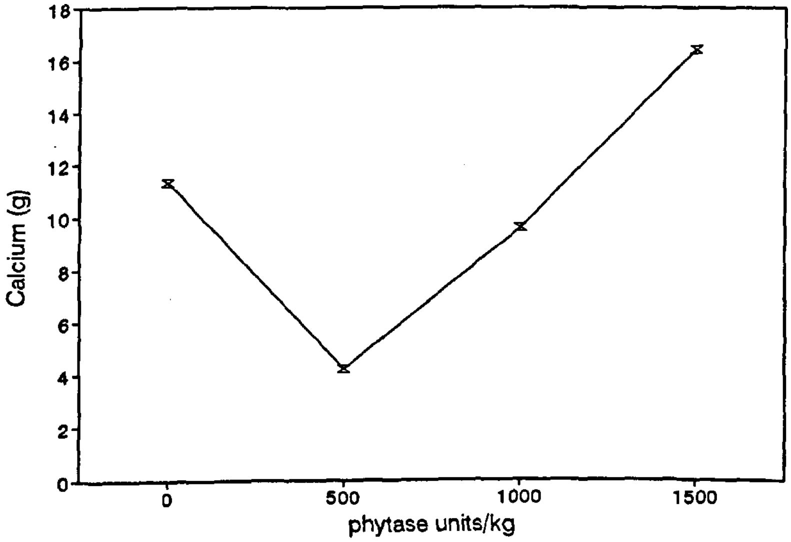


Table 5.7 Effect of enzyme and period on ash digestibility

Phytase g/kg	Basal	500	1000	1500	p	sed
	0.490	0.553	0.500	0.577	ns	0.041
Period	I	II	III	IV	p	sed
	0.599	0.560	0.518	0.442	<0.001	0.036

Table 5.8 Digestibility of nitrogen, energy and dry matter

Nutrient	Basal	+500	+1000	+1500	p	sed
Nitrogen	0.85	0.86	0.85	0.85	ns	0.009
Gross energy	0.85	0.86	0.85	0.87	ns	0.009
Digestible energy	14.75	14.85	14.78	15.07	ns	0.155
Dry matter	0.85	0.85	0.83	0.86	0.02	0.010

5.4 Discussion

5.4.1 Phosphorus balance

Addition of phytase resulted in an increase in phosphorus digestibility up to a maximum of 6 percentage units, this was lower than the 15 percentage unit increase obtained in the previous experiment, and lower than the increases reported by previous workers (Simons *et al.*, 1990; Jongbloed *et al.*, 1992; Ketaren *et al.*, 1993, Pallauf *et al.*, 1992 and Eekhout and de Paepe, 1992) who achieved improvements in apparent phosphorus digestibility ranging from 20-30% units. However, Young *et al.* (1992) achieved an increase in phosphorus digestibility of only 11 percentage units with a level of 1000 unit/kg. A summary of phosphorus digestibility responses to phytate is given in table 5.9.

Although there was a slight decrease in faecal phosphate output with phytase, this was not significant. Total faecal phosphorus output throughout the 5-day period averaged 17.6g without phytase, and 16.6g with phytase. Previous workers have obtained large reductions in faecal phosphorus output when phytase was used (eg Ketaren *et al.*, 1993 achieved a decrease from 22.5 to 14.4 g/kg; Simons *et al.*, 1990 found a decrease from 21 to 13.6g/kg; Lei *et al.*, 1993 reported a decrease from 20 to 11g/kg).

The relatively small difference in total faecal phosphorus output obtained with phytase in the present experiment is difficult to interpret since the concentration of phosphorus in the faeces was significantly lowered with the enzyme. The increase in total faecal phosphorus output across the periods was consistent with results of the previous trial, and is further evidence that the phosphorus content of the diet should be lowered in several phases as the pig nears its finishing weight. This increase is also interesting in that it confirms suggestions of phosphorus regulation at the gut level (Gutte *et al.*, 1961; Vemmer, 1982). As the pig nears its mature size, the daily amount of dietary phosphorus increases due to an increased daily feed intake. It may be that a 'saturation point' of the intestinal membrane transport system is reached, after which excess phosphorus must be excreted via the faeces. This would differ from the more finely controlled regulatory system of calcium homeostasis. On the other hand, the declining phosphorus digestibility with increasing age may be due simply to an effect of age on the physiological digestive processes, for example, a decline in the efficiency of the transport system as the pig ages.

Contrary to the results obtained in this experiment, Lei *et al.* (1993) found no interaction of time with treatment when different levels of phytase were used, suggesting that any changes in phosphorus requirement with time were not large enough to confound the phosphorus balance. Likewise, Kemme and Jongbloed (1992) concluded that digestibility of phosphorus was not affected by the age or weight of pig in the range of 30 to 70kg.

The increase in urinary phosphorus excretion with increasing phytase (figures 5.2 and 5.3) clearly demonstrates the homeostatic mechanisms which regulate phosphorus level in the pig. The animals receiving the unsupplemented basal diet excreted urine containing a low phosphorus content. Upon addition of phytase to the diet, which caused a greater amount of phosphorus to become available to the animal, renal regulatory mechanisms acted to shunt some of this extra phosphorus into the urine. The extent of fine-tuning of this mechanism is unknown, but on the basis of the current results it would appear to be quite sensitive.

As the level of dietary phosphorus effectively increased incrementally due to phytase additions, a greater amount of phosphorus was excreted into the urine. Because the concentration of urinary phosphorus increased even at the lowest level of phosphorus (500 units/kg) it could be assumed that a low level of phytase caused release of phosphorus sufficient to meet the animals' requirements. There was a further increase in urinary phosphorus excretion at 1500 units/kg, although from the digestibility data a slight decrease in urinary phosphorus excretion would have been expected.

Table 5.9 Comparison of present and published responses to phytase

Source	Diet	% unit increase in P digestibility
Simons *et al.*, 1990	Maize-soya	26
	Tapioca-hominy feed	22
Jongbloed *et al.*, 1992	Maize-soya	30
	Tapioca-hominy feed	27
Leunissen *et al.*, 1992	Maize-soya	11
Eekhout and de Paepe, 1992	Maize-soya	19
Pallauf *et al.*, 1992	Maize-soya	35
Ketaren *et al.*, 1993	Artificial (sucrose)	24
Present results	Maize-soya	6

Upon addition of phytase to the diet, urinary phosphorus excretion counteracted phosphorus intake to such an extent that the amount retained in the body remained almost constant. This was surprising since an increase in retention would have been expected between the basal diet and those with added phytase. Lei *et al.* (1993) observed that pigs fed phytase retained 50% more phosphorus daily than control pigs. These workers used a total phosphorus level of 0.32%, with no supplementary inorganic phosphate, thus diets were deficient to a greater extent than in the present experiment. Almost a complete retention of phosphorus was achieved with both control and phytase-supplemented diets, and with the latter, there was an almost identical increase in apparent digestibility and retention (table 5.10). There was only a marginal effect of the enzyme on the proportion of phosphorus retained to that digested, indicating that the increase in retention due to phytase were solely due to an increased digestibility, rather than an increased retention rate *per se.*

It is assumed that in the present experiment the addition of phytase caused an excess of phosphorus which was then excreted to keep the amount retained almost constant. This was further illustrated by the amount of phosphorus retained as a proportion of that digested, which decreased with increasing phytase additions (p= 0.002, sed= 4.14; figure 5.7). Phosphorus retention, at more than 4g/day, was relatively high. Data of Lei *et al.* (1993) are presented for comparison in table 5.11.

It seemed apparent from current observations that the basal diet was almost sufficient to meet requirements for phosphorus. Animals in metabolism crates grow more slowly than under commercial conditions, and thus would be expected to have lower daily requirements for phosphorus.

Figure 5.7 - Retained phosphorus as a percentage of digested phosphorus

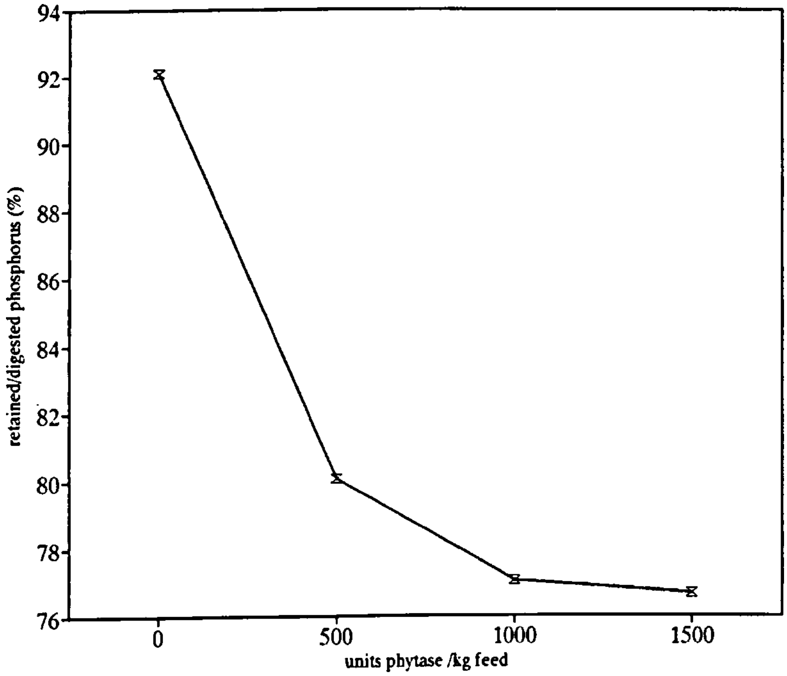


Table 5.10 **Effect of phytase on phosphorus retention of growing pigs (data of Lei *et al.*, 1993)**

Phosphorus	- phytase	+ phytase
Retained (g/day)	2.046	2.228
As % of intake	54.5	69.3
As % of digested	76.9	82.9

Table 5.11 **Effect of increasing phosphorus level on phosphorus balance (data of Lei *et al.*, 1993 and present experiment)**

	Lei *et al.*, 1993 (weanling pigs)		Present results (growing pigs)	
	0.29% P	0.62% P	0.5%P	+ phytase[*]
Intake mg/day	1092	1618	8710	8930
Retained (% of intake)	27	50	56	50
Retained (% of digested)	99	95	92	77
Digestibility	0.28	0.53	0.59	0.65
Faecal mg/kg	792	763	176	165
Urinary mg/kg	3	39	11	43

[*] 1000 units phytase/kg feed

Mroz *et al.* (1994) considered 0.5% total phosphorus to be close to the requirement of growing pigs. Had a lower level of phosphorus in the basal diet been used, addition of phytase would have been expected to result in a greater retention. On the other hand, because of the uncertain efficacy of phytase at low inclusion levels, it seemed prudent to provide a level of phosphorus that would not cause a severe deficiency in the event that no extra phosphorus was made available, particularly as animals undergoing metabolism trials are subject to rigorous welfare regulations.

The diet was formulated to provide a relatively high level of phytate phosphorus with an equivalent amount of non-phytate phosphorus. The phosphorus level used in the experimental diet was approximately 5g/kg. Of this it was presumed that 2.1g/kg was present as phytate-phosphorus and was indigestible without the addition of phytase. It was intended that the diet should remain deficient in phosphorus even if some phytate-phosphorus was released. But it seems that the requirement may have been overestimated, either because of the slower growth of these animals compared with those under commercial conditions, or because the recommended requirements for growth given by the ARC (1981) are too high. A lower level of phosphorus in the basal diet would have been made possible by excluding the dicalcium phosphate.

It has been proposed that smaller increases in digestibility would be obtained at higher dietary levels of phosphate (Lei *et al.*, 1993). However, whether or not dietary phosphorus influences phosphorus transport across the gut is questionable. Earlier work by Whittemore (1973) suggested that phosphorus transport across the gut, unlike that of calcium, was mainly independent of phosphorus level. Inorganic phosphate is a strong inhibitor of phytase activity (Shieh and Ware, 1968; Gibson and Ullah, 1988) and in this experiment this effect may have come into play. However, there was a relatively large increase in digestibility at the lowest level of phytase (500 units/kg) and if there was an inhibitory effect it would have been expected that this would have been seen here. This was not the case - it was at higher levels of phytase that no additional benefits were seen.

5.4.2 Shape of the response

The relationship between apparent phosphorus digestibility and phytase level could be described with a quadratic curve, as previously presented in figure 5.4. The response was in contrast to the exponential curve obtained using 6 levels of phytase from 0 to 2000 units/kg in a maize-soybean diet for growing pigs (Beers, 1992). However, the latter results also showed a rapid increase in digestibility at levels of up to 400 units/kg, and thereafter a diminishing increase. The magnitude of response of the present experiment was similar to that obtained by Young *et al.* (1993) who fed 0, 500 and 1000 units/kg to piglets, and obtained phosphorus digestibility coefficients of 0.63, 0.71 and 0.74 respectively. Present results may also have fitted a "broken stick" response, but since only four levels of phytase were used, the point of inflection would be difficult to determine. A broken stick response would have implied that at a certain discreet level of phytase the response reached a maximum, and that up to this point the increase in digestibility of phytase was the same for each unit of phytase, whereas the quadratic model implied that as the phytase built up the effects of the enzyme became limiting.

Given the known physiological mechanisms of enzyme inhibition, the latter model seemed more realistic. In terms of biological response, it known that phosphorus uptake is not finely regulated (with respect to dietary phosphorus intake) at the level of the gut in pigs. Thus the changes in apparent digestibility reflect changes in availability of phosphorus in the gut and in this case, could be attributed to enzyme activity in the gut. There may be a cumulative 'limiting' effect of the enzyme either directly by end-product inhibition or indirectly by altering the gut environment which could become unfavourable to the enzyme.

The equation of the curve was $y = 0.5832 + 0.000162x - 0.84E^{-7}x2$ ($p=0.048$; y=apparent phosphorus digestibility, x= phytase units/kg feed). From this it could be estimated that the maximum response would be achieved using 900-1000 units phytase per kg. However, a large response obtained at levels of up to 400 units/kg, followed thereafter by a diminishing increase (table 5.12) suggested that 400 -500 units/kg may be a more prudent dosage to use.

When the response of weanling pigs to graded levels of phytase (0, 250, 500 and 750 units/kg) was measured (Lei *et al.*, 1993) it was found that plasma phosphate concentration increased linearly with dietary phytase addition. There was no maximum break point of plasma phosphate concentration; indeed, linear effects of phytase activity up to 750 units/kg were very consistent. Similar response patterns in chicks to supplementary phytase have been reported by Nelson *et al.* (1971) and Schoner *et al.* (1991). However, it is unlikely that the response would be linear at higher levels of phytase. Indeed, it would be expected that, if the 'cumulative limiting effect' does apply, the enzyme would become limiting at a lower level than with pigs because of the effectual separation of the crop from the rest of the gastro-intestinal tract in birds.

In pigs, growth and bone strength increased linearly with phytase level up to 1000 units/kg (Cromwell *et al.*, 1993). Again, a higher level of phytase was not used, and it would seem unlikely that the response would continue linearly. Based on urinary phosphate output, which is a recognised indicator of phosphorus adequacy, the present work suggested that even at low levels (400-500 units/kg) of phytase enough phosphorus was released to satisfy the requirements of the animals under these conditions. This is lower than the optimum level of (1000) units/kg level suggested by Simons *et al.* (1990). However, animals were slow-growing in comparison to those under commercial conditions, thus it is probable that their nutrient requirements were also lower. On the other hand, given the relatively high costs of enzyme supplementation, a level of 400-500 units/kg, while not attaining maximum release of phytate-phosphorus, may be sufficient to satisfy the phosphorus requirements of growing pigs.

Table 5.12 **Incremental response of phosphorus digestibility to phytase level ($y = 0.5832 + 0.000162x - 0.84E^{-7}x2$)**

Units phytase/kg feed	0	100	200	300	400	500	600
Phosphorus digestibility	0.583	0.599	0.612	0.624	0.639	0.643	0.650
Change in digestibility	0	0.016	0.013	0.012	0.011	0.008	0.007
Units phytase/kg	700	800	850	900	950	1000	1100
Phosphorus digestibility	0.655	0.659	0.660	0.661	0.661	0.661	0.660
Change in digestibility	0.005	0.004	0.001	0.001	0.000	0.000	-0.001

5.4.3 Calcium balance

The lack of change in calcium digestibility due to enzyme addition was consistent with results of the previous experiment using cannulated pigs, but in contrast to increases in calcium digestibility which have previously been reported by Nasi (1990) and Simons *et al.* (1990). Lei *et al.* (1993) found that pigs receiving phytase achieved a 13% increase in apparent digestibility and a 14% increase of retained calcium as a proportion of intake. Daily faecal output of pigs fed phytase was reduced by 52% whereas daily urinary calcium output was not altered. These workers suggested that phytase may increase calcium utilisation indirectly by an increased phosphorus utilisation, because dietary calcium is utilised for skeletal growth only when dietary phosphorus is simultaneously utilised.

The decrease in urinary calcium concentration from the basal diet to that containing 500 phytase units/kg, and to a lesser extent from the basal diet to that containing 1000 phytase units/kg, was similar to the decrease in urinary calcium obtained when dietary phosphorus level was raised (Mroz *et al.*, 1994). However, at the highest level of phytase (1500 units/kg) urinary calcium excretion exceeded that of the basal diet. In view of these results, the effect of phytase on urinary calcium concentration could be interpreted as a phosphorus level x calcium level interaction. Initially, diets were deficient in phosphorus, but addition of dietary phytase caused release of phosphorus so that the calcium: phosphorus ratio became more favourable for apatite formation, and thus urinary calcium excretion was lowered. However, as the level of phytase increased to 1500 units, the ratio of calcium: phosphorus became unfavourable, this time due to an excess of phosphorus, and calcium was excreted via the urine.

An evaluation of the validity of this proposed mechanism must also consider previous work by Hansard *et al.* (1961) and Miller *et al.* (1962, 1964), which indicated little effect of dietary phosphorus on urinary calcium excretion. On the other hand, Mroz *et al.* (1994) found that urinary calcium excretion was low (3-17 mg/kg compared to 100-150 mg/kg) when dietary phosphorus level was greater than or equal to calcium level, and increased as dietary calcium exceeded the level of dietary phosphorus.

Under practical conditions, urinary calcium losses are estimated to be around 1% of daily intake (Pointillart, 1991). Approximately 50% of the total calcium in the urine is ionized (Calvo *et al.*, 1982), the remainder being complexed with anions such as phosphates, citrates, sulphates and oxalate. Since these calcium complexes are not readily absorbed, an increase in plasma or renal phosphorus would be expected to increase the total calcium excretion in the urine. This may interact with the improved ratio causing an enhanced uptake.

In the present experiment, addition of phytase effectively lowered the calcium to phosphorus ratio from 1.46 to 1.38; this may have confounded any effects of the enzyme on calcium and/or phosphorus digestibility. Vipperman *et al.* (1974) proposed that the utilisation of phosphorus was affected by the calcium to phosphorus ratio to a greater extent than the utilisation of calcium, but whether or not the calcium to phosphorus ratio contributed to the overall values of phosphorus digestibility is unclear. *In vitro* studies of Krol-Kramer (1992) found inhibiting effects of elevated calcium supply on the liberation of orthophosphates, particularly at levels above 7g/kg. Mroz *et al.* (1994) looked at the influence of graded calcium supply on microbial phytase efficacy in starter diets at a constant phosphorus level of 4.3 g/kg, of which phytate phosphorus was 2.1-3.4 g/kg. They found that calcium and phosphorus content of the urine, was influenced by interactions between calcium level and phytase dose, and proposed a level of 0.75% calcium and 0.5% phosphorus.

It is generally considered that calcium to phosphorus ratio becomes important only at low levels of phosphorus. In order to overcome the changing ratio it would have been necessary to adjust the calcium level with each level of phytase, but this would have required knowledge as to the amount of phosphorus being made available by the enzyme. Alternatively, a range of calcium levels could have been used at each dietary phytase level, which would either require more animals in total (which was impractical) or fewer animals per treatment (which would have lowered the statistical power of the experiment).

5.4.4 Digestibility of ash

Although there was a lack of significant effect of the enzyme on overall digestibility of ash (any slight differences probably being due to changes in the digestibility of phosphorus) there was a strong period effect, with ash digestibility decreasing steadily from the first to the last period. This may have been partly an effect of phosphorus digestibility, but is likely to have been mainly due to the declining calcium digestibility from the first to the last period, which was somewhat more apparent.

5.4.5 Nitrogen, energy and dry matter

The lack of effect of phytase on nitrogen digestibility was surprising since the previous trial indicated an increase in ileal and total tract digestibility with the enzyme. This discrepancy is further confused by findings of Ketaren *et al.* (1993) that an increased protein retention with the enzyme occurred only when animals were deficient in phosphorus. It may be that an effect of the enzyme on nitrogen digestibility can only be expected if the phosphorus level remains deficient. Earlier assumptions of the enzyme cleaving phytate-protein bonds may need to be modified, since this would be independent of the phosphorus level.

The lack of enzyme effect on digestibility of dry matter or energy was in accordance with the previous trial, confirming that the enzyme was not removing the inhibitory effect of phytate on alpha amylase. The latter enzyme is active proximal to the stomach, and it could be assumed that phytate hydrolysis takes place too late to allow disinhibition of alpha amylase.

5.4.6 Efficacy of the phytase used

Since the optimum suggested level of phytase inclusion had previously been assumed to be 1000 units/kg, it seemed reasonable in this experiment to use a level of phytase either side of this suggested level. Alternatively, 6 levels of phytase could have been used, with 300-unit increments, but since the inclusion level at the lowest level of phytase would have been less than 1g/kg, thorough mixing would have been more difficult to ensure. More importantly, in order to discriminate differences with an 80% degree of certainty, a minimum of 12 animals per treatment was needed (based on 10% expected coefficient of variation. Thus 0, 500, 1000 and 1500 phytase per kg of feed seemed to be the most appropriate levels to use.

The phytase assay used measured the amount of free orthophosphate liberated from phytate by phytase. Very recently, it has been suggested (TNO Netherlands, pers. communications, 1994) that this test is insufficient, as it does not give an indication of the degradation pattern, and that high performance liquid chromatography, which enables measurement of intermediate products of hydrolysation, is more appropriate. However, while partial hydrosylates of phytic acid are powerful inhibitors of calcification *in vitro* (Thomas and Tilden, 1972), there is no evidence to indicate that these can be absorbed from the gut in pigs. It was assumed for our experiments that as only the free orthophosphate could be utilised, therefore the sodium phytate test, which measures free orthophosphate, was considered appropriate. Despite this, HPLC analysis may be useful to discriminate between a so-called 'potent' phytase, ie one that degrades phytate completely to free orthophosphate, and a phytase that may show the same resultant activity, but which produces a large number of intermediates without further hydrolysis to inorganic phosphate.

When 1000 units/kg phytase was used, the 6% unit increase in phosphorus digestibility enabled 14.2% of the phytate-phosphorus to be digested (table 5.13). This was lower than the estimate of Cromwell *et al.* (1993) who fed graded levels of phytase with a corn-soybean diet and concluded that 1000 units/kg converted approximately one third of the phytase phosphorus to an available form.

The increase in digestibility obtained at a phytase level of 1000 units/kg in the present experiment was also smaller than obtained in the previous experiment at a similar inclusion level, using similar sized pigs of the same sex. The explanation may have lay in the different diets used; the previous diet used a barley-based diet whereas maize was the main cereal used in the present experiment. In a similar experiment with piglets, the increase in apparent phosphorus digestibility obtained by addition of 1000 units phytase/kg was greater when a maize-based diet was fed than with a wheat-based diet (Eekhout and de Paepe, 1992). It has been suggested (Jongbloed *et al.*, 1993) that the phytase dose response depends on the source and amount of phytate in the diet. These authors found that maximal efficiency of 500 units/kg feed was obtained up to 1.2 g phytate/kg feed. The relatively high level of inorganic phosphate used in the present trial may have exerted a negative influence on the activity of the phytase. But despite the lower increases obtained in the present experiment, addition of phytase still released a substantial amount of phytate-phosphorus for digestion. This work therefore gave strong indications that a lower level of phytase than previously assumed could be used efficaciously to improve phosphorus digestibility. Given the relatively high cost of the enzyme, use of 500, rather than 1000 units/kg may make it a cost-effective alternative to phosphates, particularly in areas of intensive livestock production, where pollution is prohibitive.

5.5 Conclusions

The response of pigs fed a high-phytate diet to phytase could be described as :

$$y = 0.583 + 0.00016x - 0.84E^{-7}x^2$$

where y = phosphorus digestibility
x = units phytase/kg feed

From this it was concluded that the maximum increase in apparent phosphorus digestibility was achieved at an inclusion rate of 900-1000 phytase units/kg. However, a level of 400 - 500 units/kg also gave a relatively large increase in digestibility and in economic terms would be the sensible dose rate to use.

Table 5.13 Calculation of phytate-phosphorus made digestible by addition of 1000 units phytase/kg feed

P digestibility without phytase	0.59
Digestible P without phytase	2.96 g/kg
P digestibility with phytase	0.65
Digestible P with phytase	3.26 g/kg
Assuming extra P digested is of phytate origin	
Percent of phytate P made digestible	(3.26 - 2.96/2.12) * 100 =14.2%

Chapter 6 Experiment 3 - Effect of phosphorus and phytase on growth and bone development of young pigs

6.1 Introduction

The dietary phosphorus requirement of young pigs is not well established. Values of a net requirement estimated by the ARC (1981) extend from 2.7 g/day for 5kg liveweight pigs to 4.6 g/day for 25 kg liveweight pigs, but figures obtained from the literature deviate somewhat from this. The discrepancy occurs when attempts are made to define requirement in terms of dietary intake. Although there is general agreement as to the daily dry matter intake, estimates of phosphorus availability differ markedly. The NRC (1988) defines its requirements on the assumption that at least 30% of dietary phosphorus is of plant or animal origin. Daily recommended phosphorus intakes are 3g for 5-10kg pigs and 7.5g for 25kg pigs, or 6.5-6.0 g/kg freshweight. The ARC (1981) suggested dietary levels of 9.2-5.9 g/kg were based on 70-80% availability which may be unrealistic.

Brown *et al.* (1972) showed that maximum bone formation occurs within the first twelve weeks of life. Although compensatory mineralization has been shown to occur, effects on later development are unknown. Thus, when defining a requirement for pigs of this size it must be considered that although maximum bone mineralization is not necessary for optimum growth performance, later bone development must not be impaired by a mineral deficiency. Bone strength has been used as an indicator of mineral availability. Crenshaw *et al.* (1981) found that femur strength was responsive to dietary phosphorus level in pigs between 2 and 4 months of age, whereas strength of the metacarpal and metatarsal bones were more sensitive indicators in older pigs.

It may be possible supplement phytase into phytate-rich diets for young pigs, thus reducing the need for inorganic supplements. Use of the enzyme in piglet diets has been attempted (Beers and Jongbloed 1992; Hoppe *et al.*, 1993; Eekhout and de Paepe, 1992) resulting in an increased growth performance over and above that which would be expected by an increased phosphorus availability alone.

By establishing a response both with and without phytase at increasing levels of dietary phosphorus, it may be possible to calculate the amount of phosphorus made available by the phytase enzyme. The following experiment was therefore conducted to clarify the requirement for phosphorus in piglets, and to quantify the amount of phosphorus made available by the use of phytase.

6.2 Materials and Method

6.2.1 Experimental Design

9 levels of non-phytate phosphorus were achieved by incremental addition of inorganic phosphorus to a basal diet. Each diet was fed either with or without phytase, to give a total of 18 diets, each of which was fed *ad libitum* to 4 piglets (2 male, 2 female).

6.2.2 Diet

The diet formulation and nutrient specifications are shown in tables 6.1 and 6.2 respectively. The basal diet contained a low level of non-phytate phosphorus (2 g/kg; assumed to be readily digestible) with an equivalent amount of phosphorus present in the form of phytate phosphorus. 9 levels of dietary phosphorus were achieved by the addition of mono ammonium phosphate to the basal diet. A summary of the diets is given in table 6.3. Each of the 9 dietary levels of phosphorus were fed either with or without phytase added at a level of 1000 units/kg freshweight.

Table 6.1 **Formulation and phosphorus/phytate content of basal diet**

Ingredient	g/kg	phosphorus (g/kg FW)	Total phosphorus contribution to diet	Phytate-phosphorus (%)	Phytate-phosphorus contribution to diet
Maize	385	2.6	1.00	66	0.66
Wheat	185	3.3	0.60	73	0.44
Barley	50	3.9	0.20	64	0.13
Rapeseed meal	23	6.7	0.15	35	0.05
Soya 50	180	6.8	1.22	61	0.75
Soya oil	50	-	-	-	-
Dried skim milk	100	10	1.0	-	-
Betamix 314	12.5	-	-	-	-
Limestone	7.0	-	-	-	-
Lysine	4.0	-	-	-	-
Methionine	1.0	-	-	-	-
Threonine	2.0	-	-	-	-
Tryptophan	0.5	-	-	-	-
Total	1000		4.17		2.02

Table 6.2 Nutrient specification of basal diet

Nutrient	g/kg Freshweight
Dry matter (%)	89.0
Digestible energy (MJ/kg)	15.59
Crude protein	195.37
Oil	72.65
Crude fibre	25.25
Lysine	13.66
Methionine	4.30
Methionine + cystine	7.49
Threonine	9.02
Tryptophan	2.28
Phenylalanine + tyrosine	16.45
Ash	37.03
Calcium	7.89
Phosphorus	4.17
Salt	4.47
Linoleic acid	37.31

Table 6.3 Phosphorus and phytase of experimental diets

Diet	g/kg non-phytate P	g/kg total P	+/- phytase
1	2.0	4.2	-
2	2.5	4.7	-
3	3.0	5.2	-
4	3.5	5.7	-
5	4.0	6.2	-
6	4.5	6.7	-
7	5.0	7.2	-
8	5.5	7.7	-
9	6.0	8.2	-
10	2.0	4.2	+
1	2.5	4.7	+
12	3.0	5.2	+
13	3.5	5.7	+
14	4.0	6.2	+
15	4.5	6.7	+
16	5.0	7.2	+
17	5.5	7.7	+
18	6.0	8.2	+

6.2.3 Procedure

The trial was conducted according to the procedure in *Section 3.5.3* of the General Materials and Methods. Piglets were assigned randomly within sex to one of the 18 diets. Growth performance was monitored from 10-25 kg liveweight after which pigs were slaughtered. The carcase with blood and offal was ground, and samples of the ground tissue analyzed for ash, phosphorus and calcium percent in the dry matter (DMAsh%, DMP%, DMCa%). Total ash, phosphorus and calcium (Ash, P, Ca) were calculated by multiplying the sample percent by the dry weight of the pig. Ash, calcium and phosphorus content of the freshweight (FWAsh%, FWCa%, FWP%) were obtained by dividing total ash, P and Ca by the freshweight of the whole body.

Physical measurements of the right hind femur and the right third and fourth metatarsals (freshweight, longitudinal length and breaking strength were determined on the fresh bones. Thereafter ash, phosphorus and calcium content of the fat-free bones were evaluated (Ash%, P%, Ca%); these were multiplied by the fat free weight of the bones to obtain total ash, phosphorus and calcium.

6.2.4 Statistical Analysis

Growth, feed intake and feed conversion efficiency data were subjected to analysis of variance. Analysis of covariance using 'slaughterweight' as the covariate was performed for bone data and carcase composition data. For the analyses of variance and covariance, 'level of non-phytate phosphorus in the diet' (g/kg freshweight), phytase, and gender were used as treatments. Bone physical measurements (freshweight, length, strength), bone mineral content (total ash, ash%, total phosphorus, P%, total calcium, Ca%) and carcase mineral data (ash, P, Ca, DMAsh%, DMP%, DMCa%, FWAsh%, FWP%, FWCa%) were regressed against daily intake of non-phytate phosphorus and daily intake of total phosphorus. Finally, correlations between bone strength and bone mineral content, bone strength and carcase mineral content, and between carcase mineral content and bone mineral composition were investigated using linear and non-linear regression analyses.

6.3 Results

6.3.1 Health of the pigs

Throughout the trial some scouring problems occurred; these were treated with the antibiotic depocillin. All pigs treated recovered fully to complete the trial. No locomotory problems were seen.

6.3.2 Growth performance

Growth performance of individual animals is presented in appendix III. The effects of phosphorus level, phytase, sex and interactions on feed intake, daily liveweight gain and feed conversion efficiency are summarised in table 6.4. It could be seen from the sed value that there was an effect of enzyme between diets 1 (basal diet; 2g non-phytate phosphorus/kg) and diet 10 (basal diet + phytase), despite the non-significant probability value obtained from analysis. As the probability values for the effects of enzyme attained by this analysis were mean values across all phosphorus levels, further analysis of enzyme effect between diets 1 and 10 was carried out; the results of which are shown in table 6.5.

A lowered feed intake of pigs receiving the diet containing 2g/kg non-phytate phosphorus (mean 775 g/day) was compensated for by the addition of phytase (mean 902 g/day; $p = 0.085$) and approached that of pigs on the 4g/kg non-phytate phosphorus diet (mean 925 g/day; figure 6.2). These results were reflected in the daily liveweight gains which showed a similar trend although the differences here were statistically non-significant ($p = 0.121$; figure 6.1). There was no difference in feed conversion efficiency across the treatment groups (figure 6.3) Mean feed conversion ratio was 1.48. Growth rate was not different between sexes, but boars had lower feed intakes and a better feed conversion efficiency than gilts.

Regression analysis revealed strong linear and quadratic responses of daily feed intake and daily liveweight gain, both to daily non-phytate phosphorus intake and to daily total phosphorus intake (table 6.6). Addition of phytase destroyed the response of average daily gain to phosphorus intake, and weakened the response of feed intake to phosphorus intake. No relationships between feed conversion efficiency and phosphorus intake were apparent, either with or without phytase.

Table 6.4 **Significance of effects of phosphorus level, phytase, sex and interactions on growth, feed intake and feed conversion efficiency**

	feed intake		*liveweight gain*		*feed conversion ratio*	
	p	sed	p	sed	p	sed
P level	ns	0.048	ns	0.035	ns	0.059
phytase	ns	0.022	ns	0.016	ns	0.028
sex	0.053	0.022	ns	0.016	0.032	0.028
P level * phytase	ns	0.067	ns	0.049	ns	0.083
P level * sex	ns	0.067	ns	0.049	ns	0.083
phytase * sex	ns	0.031	ns	0.023	ns	0.039
P level * phytase * sex	ns	0.095	ns	0.070	ns	0.012

Table 6.5 **Significance of effect of adding phytase to the basal diet**

	Treatment means			
	- phytase	+ phytase	P	sed
Liveweight gain	0.507	0.604	0.121	0.057
Feed Intake	0.775	0.902	0.085	0.056
Feed conversion ratio	1.545	1.500	0.393	0.047

Figure 6.1 Effect of phosphorus level and phytase on daily liveweight gain of young pigs

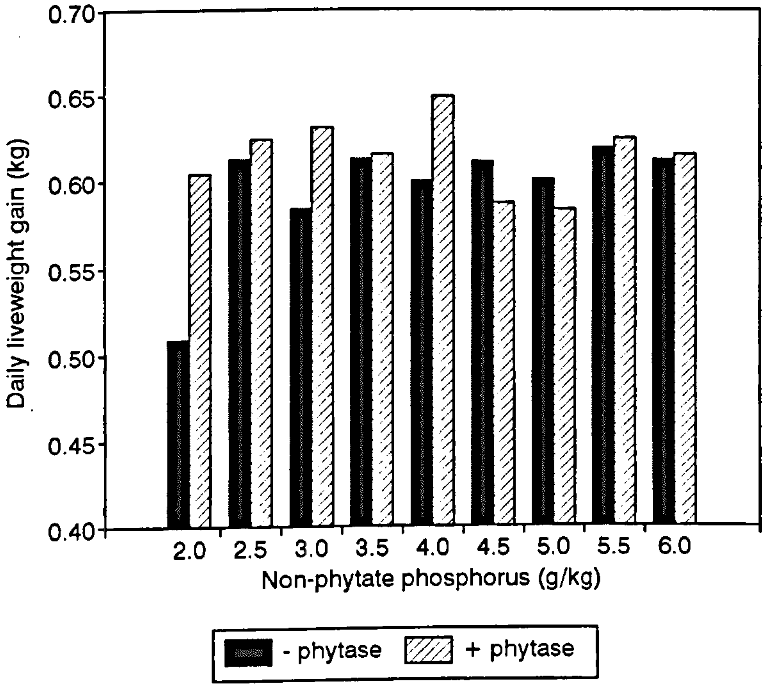


Figure 6.2 **Effect of phosphorus level and phytase on daily feed intake of young pigs**

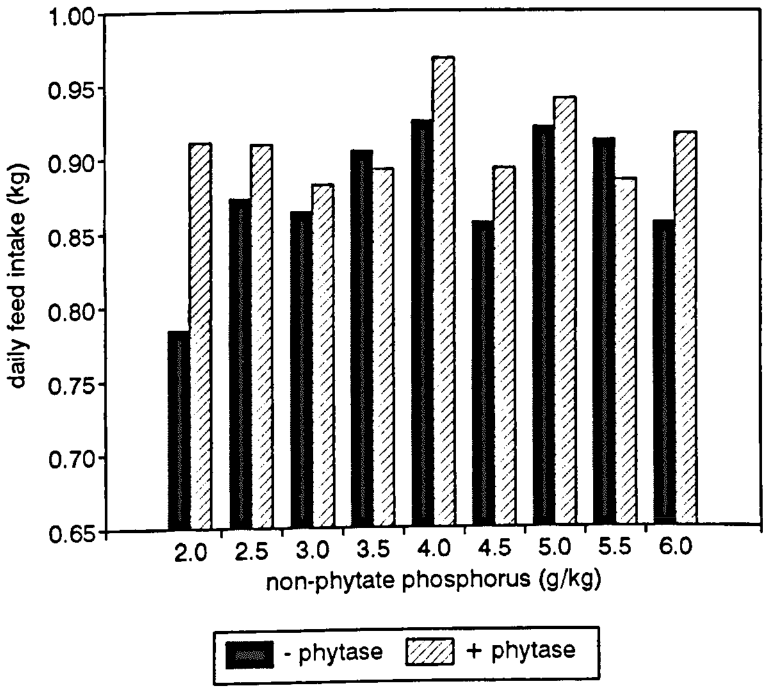


Figure 6.3 Effect of phosphorus level and phytase on feed conversion ratio of young pigs

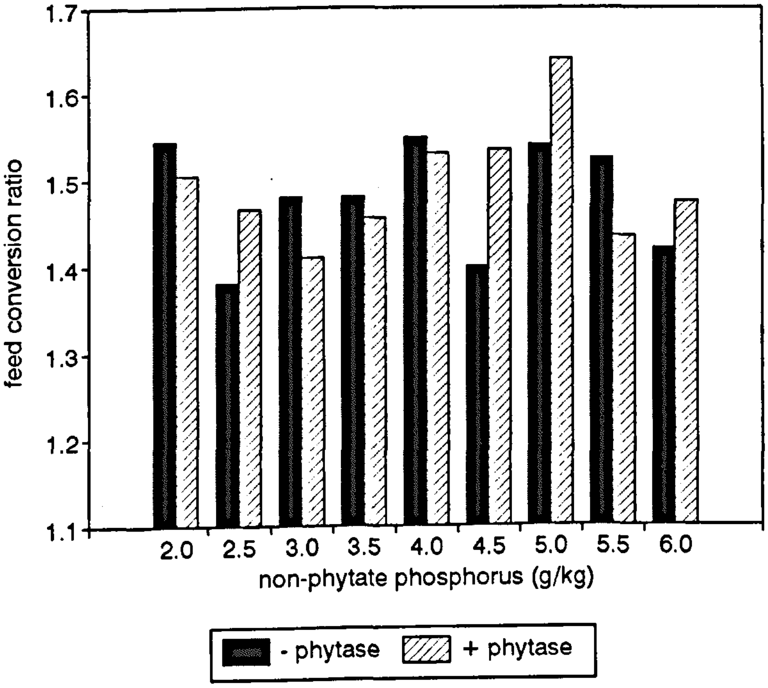
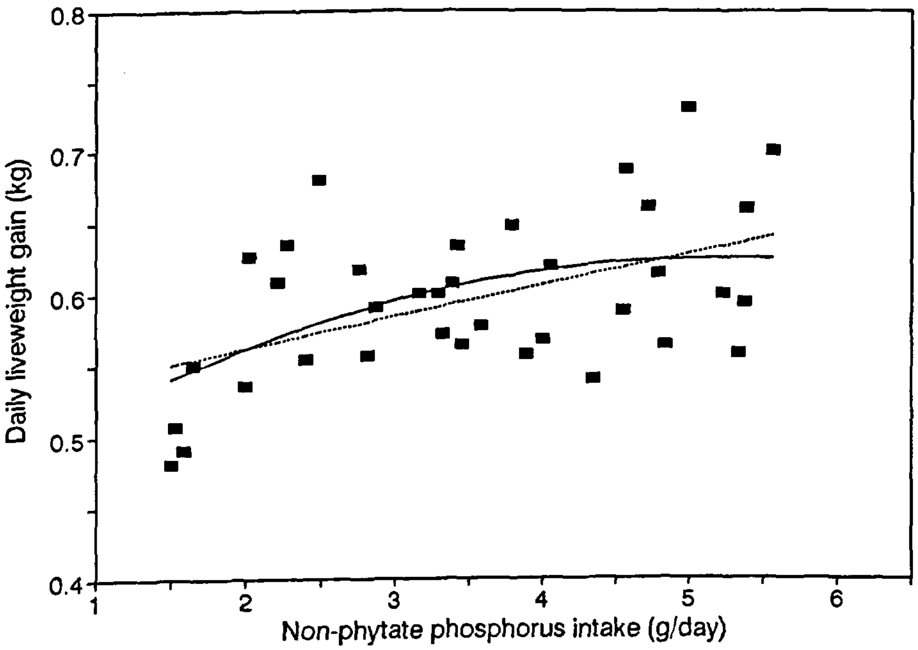


Table 6.6 Regression of growth parameters (y) against daily phosphorus intake (x)

	- phytase			*+ phytase*		
	equation of response	p	% var[τ]	equation of response	p	% var[τ]
Daily non-phytate phosphorus intake			*linear*			
daily liveweight gain	$y = 0.518 + 0.022x$	0.002	21.9	ns	>0.10	-
feed intake	$y = 0.755 + 0.035x$	0.002	22.2	$y = 0.823 + 0.025x$	0.031	10.5
feed conversion ratio	ns	>0.10	-	ns	>0.10	-
Daily total phosphorus intake						
daily liveweight gain	$y = 0.491 + 0.018x$	0.001	24.6	ns	>0.10	-
feed intake	$y = 0.701 + 0.030x$	<0.001	29.5	$y = 0.772 + 0.023x$	0.008	16.7
feed conversion ratio	ns	>0.10	-	ns	>0.10	-
Daily non-phytate phosphorus intake			*quadratic*			
daily liveweight gain	$y = 0.46 + 0.063x - 0.006x^2$	0.007	21.7	ns	>0.10	-
feed intake	$y = 0.69 + 0.080x - 0.006x^2$	0.008	21.0	$y = 0.91 - 0.029x + 0.007x^2$	0.078	9.1
feed conversion ratio	ns	>0.10	-	ns	>0.10	-
Daily total phosphorus intake						
daily liveweight gain	$y = 0.40 + 0.053x - 0.003x^2$	0.004	24.2	ns	>0.10	-
feed intake	$y = 0.60 + 0.066x - 0.003x^2$	0.002	28.2	$y = 0.92 - 0.029x + 0.004x^2$	0.023	15.5
feed conversion ratio	ns	>0.10	-	ns	>0.10	-

[τ] Percentage variance accounted for

Figure 6.4 Response of daily liveweight gain (y) to daily phosphorus intake (x)

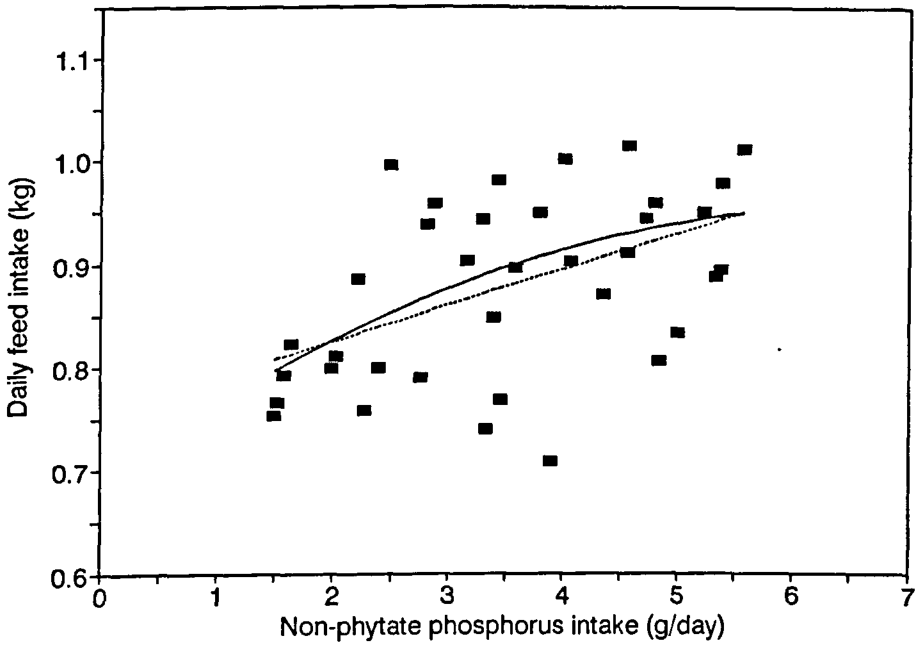


Response equations

Linear $y = 0.518 + 0.022x$ $p = 0.002$ %var 21.9

Quadratic $y = 0.46 + 0.063x - 0.006x^2$ $p = 0.007$ %var 21.7

Figure 6.5 Response of daily feed intake (y) to daily phosphorus intake (x)



Response equations

Linear	$y = 0.755 + 0.035x$	$p = 0.002$	%var 22.2
Quadratic	$y = 0.69 + 0.080x - 0.006x^2$	$p = 0.008$	%var 21.0

6.3.3 Carcase mineral composition

The effects of phosphorus level, phytase, gender and the interactions on mineral composition of the body are summarised in table 6.7. Concentration of mineral in the dry matter of the tissue appeared to be the most responsive to the treatments. Addition of phytase resulted in an increased percentage of phosphorus in the dry matter of the whole body at levels of up to 5.5 g/kg non-phytate phosphorus ($p < 0.001$); thereafter a decrease was observed (figure 6.6). Addition of phytase resulted in an increased percentage of ash in the dry matter except at 4.0 and 4.5 g/kg non-phytate phosphorus, where those animals not receiving phytase seemed to have more ash in the dry matter (figure 6.7). Boars had a higher percentage of phosphorus and ash in the dry matter than gilts ($p=0.014$, $p< 0.001$ respectively). Neither phosphorus level, phytase or sex influenced the amount of calcium in the carcase. Mean data sets for the treatment groups are presented in tables 6.8 to 6.17.

There were significant linear and quadratic relationships between daily non-phytate phosphorus intake and mineral content of the body, and between daily total phosphorus intake and mineral content of the body. Equations describing the responses are presented in tables 6.18 and 6.19. Linear and quadratic responses of DMP% to daily intake of non-phytate phosphorus are illustrated in figure 6.8. These relationships were destroyed by the addition of phytase due to altered levels of phosphorus.

Table 6.7 Significance of effect of phosphorus level, phytase, sex and the interactions on whole body mineral content

Variate	Factor	p	sed
Total phosphorus	-	ns	-
DMP%	Diet	0.041	0.045
	Enzyme	<0.001	0.021
	Sex	0.014	0.021
	Enzyme x Sex	0.071	0.030
FWP%	-	ns	-
Total calcium	-	ns	-
DMCa%	-	ns	-
FWCa%	-	ns	-
Total Ash	Enzyme	0.056	15.80
	Diet * Enzyme	0.019	48.34
DMash%	Diet	0.029	0.321
	Enzyme	0.001	0.149
	Sex	<0.001	0.149
FWash%	Enzyme	0.048	0.060
	Diet x Enzyme	0.009	0.183
Dry matter (%)	-	ns	-

Figure 6.6 Effect of phosphorus level and phytase on percent phosphorus in the dry matter of the body

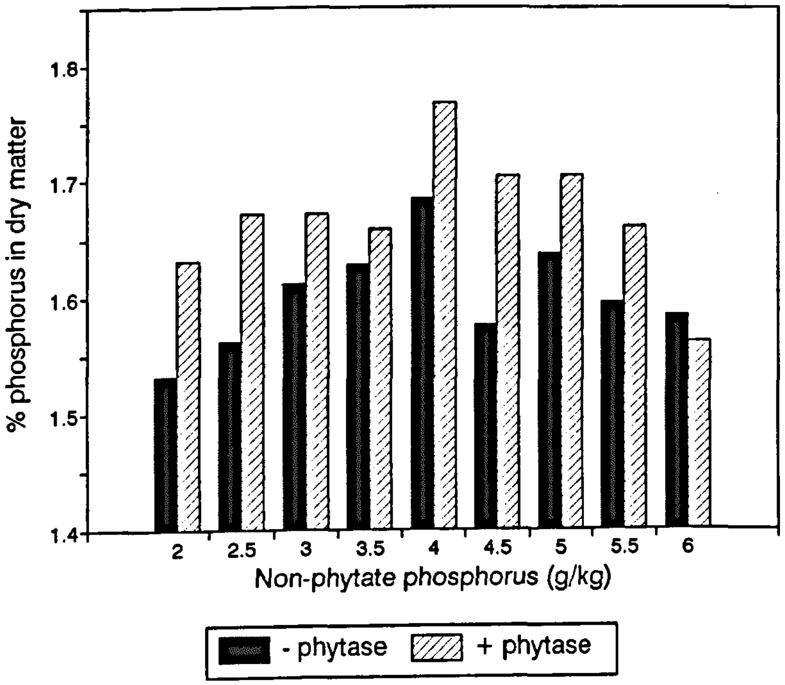


Figure 6.7 Effect of phosphorus level and phytase on percent ash in the dry matter of the body

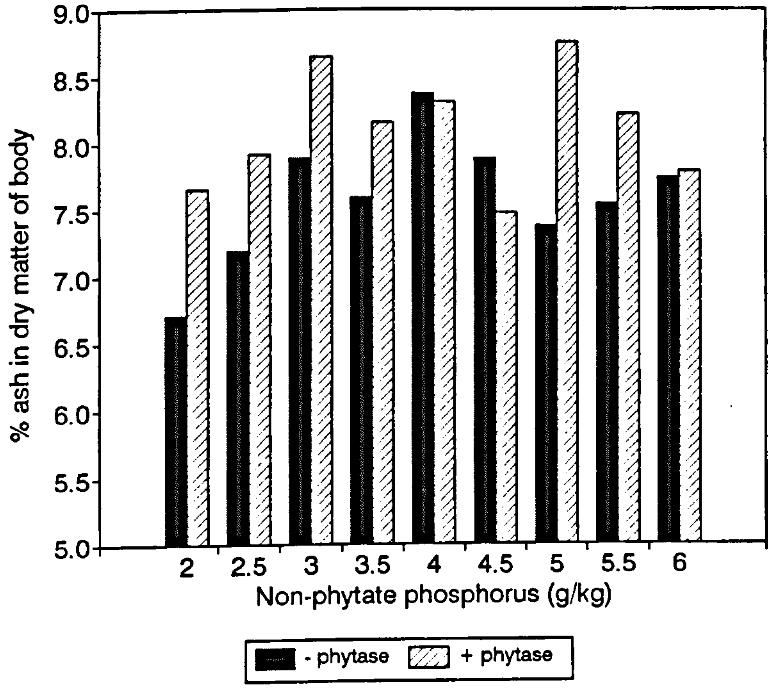


Table 6.8 Total phosphorus in the whole body - treatment group means

	- phytase		+ phytase	
	female	male	female	male
Phosphorus level[r]	*phosphorus (g) in the whole body*			
2.0	125.2	110.2	128.2	130.7
2.5	128.4	123.4	134.1	144.3
3.0	128.5	109.8	134.8	136.5
3.5	119.0	122.5	134.6	142.7
4.0	129.9	149.6	145.7	126.1
4.5	144.1	132.8	134.1	128.7
5.0	137.4	133.6	117.1	115.8
5.5	132.0	128.5	116.4	153.8
6.0	120.2	133.9	131.0	132.3

Table 6.9 Percent phosphorus in the dry matter of the whole body - treatment group means

	- phytase		+ phytase	
	female	male	female	male
Phosphorus level[r]	*% phosphorus in the dry matter*			
2.0	1.448	1.494	1.513	1.650
2.5	1.436	1.597	1.612	1.630
3.0	1.606	1.499	1.602	1.740
3.5	1.627	1.526	1.623	1.604
4.0	1.563	1.710	1.632	1.813
4.5	1.518	1.538	1.685	1.629
5.0	1.611	1.565	1.527	1.759
5.5	1.512	1.578	1.512	1.709
6.0	1.565	1.506	1.509	1.518

[r] non-phytate phosphorus g/kg freshweight diet

Table 6.10 Percent phosphorus in the fresh weight of the whole body - treatment group means

	- phytase		+ phytase	
	female	male	female	male
Phosphorus level[r]	*% phosphorus in freshweight*			
2.0	0.489	0.432	0.514	0.520
2.5	0.493	0.483	0.532	0.566
3.0	0.512	0.449	0.529	0.544
3.5	0.476	0.475	0.518	0.561
4.0	0.516	0.584	0.575	0.500
4.5	0.570	0.525	0.521	0.511
5.0	0.540	0.534	0.464	0.465
5.5	0.525	0.515	0.461	0.600
6.0	0.503	0.521	0.532	0.527

Table 6.11 Total calcium in the whole body - treatment group means

	- phytase		+ phytase	
	female	male	female	male
Phosphorus level[r]	*calcium (g) in the whole body*			
2.0	443	343	216	742
2.5	237	341	87	720
3.0	690	122	276	875
3.5	125	513	297	532
4.0	373	517	763	806
4.5	869	781	692	619
5.0	752	506	331	203
5.5	636	466	751	365
6.0	125	705	550	636

---

[r] non-phytate phosphorus g/kg freshweight diet

Table 6.12 Percent calcium in the dry matter of the whole body - treatment group means

	- phytase		+ phytase	
	female	male	female	male
Phosphorus level[r]	*% calcium in dry matter of the whole body*			
2.0	4.94	4.56	2.70	8.99
2.5	2.62	4.01	1.22	7.78
3.0	8.59	2.00	3.09	10.57
3.5	2.04	5.53	3.56	5.72
4.0	4.60	5.79	8.20	11.18
4.5	9.04	9.13	8.82	7.94
5.0	8.58	5.93	4.29	3.32
5.5	7.16	5.57	9.66	4.09
6.0	1.65	8.42	6.12	7.23

Table 6.13 Percent calcium in the fresh weight of the whole body - treatment group means

	- phytase		+ phytase	
	female	male	female	male
Phosphorus level[r]	*% calcium in freshweight of the whole body*			
2.0	1.73	1.33	0.83	2.85
2.5	0.90	1.30	0.41	2.71
3.0	2.77	0.58	1.03	3.48
3.5	0.57	1.95	1.09	2.03
4.0	1.45	1.92	2.94	3.09
4.5	3.39	3.14	2.76	2.46
5.0	2.94	2.05	1.32	0.84
5.5	2.47	1.95	2.96	1.44
6.0	0.62	2.91	2.21	2.50

[r] non-phytate phosphorus g/kg freshweight diet

Table 6.14 Total ash in the whole body - treatment group means

	- phytase		+ phytase	
	female	male	female	male
Phosphorus level[r]	*ash (g) in whole body*			
2.0	565.2	509.7	622.3	627.0
2.5	616.5	575.5	663.3	696.7
3.0	595.5	618.7	689.8	704.7
3.5	563.2	600.6	664.2	735.4
4.0	656.0	768.3	678.2	625.3
4.5	728.5	694.6	592.3	598.0
5.0	633.7	626.2	566.2	669.1
5.5	623.0	641.9	606.3	768.4
6.0	589.4	691.2	694.7	658.0

Table 6.15 Percent ash in the dry matter of the whole body - treatment group means

	- phytase		+ phytase	
	female	male	female	male
Phosphorus level[r]	% ash in dry matter of the whole body			
2.0	6.488	6.914	7.360	7.946
2.5	6.899	7.471	7.958	7.874
3.0	7.450	8.322	8.235	9.052
3.5	7.709	7.454	8.023	8.279
4.0	7.925	8.798	7.599	9.006
4.5	7.668	8.092	7.392	7.546
5.0	7.421	7.326	7.394	10.10
5.5	7.188	7.888	7.862	8.552
6.0	7.676	7.794	8.020	7.553

[r] non-phytate phosphorus g/kg freshweight diet

Table 6.16 Percent ash in the fresh weight of the whole body - treatment group means

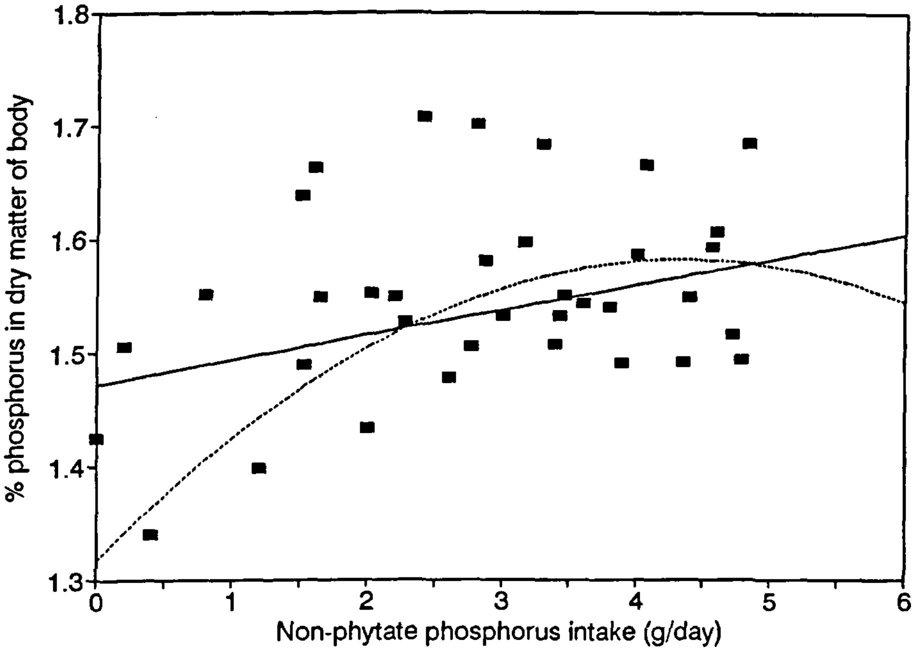
	- phytase		+ phytase	
	female	male	female	male
Phosphorus level[r]	*% ash in freshweight of whole body*			
2.0	2.194	1.996	2.495	2.506
2.5	2.373	2.244	2.629	2.734
3.0	2.369	2.499	2.714	2.815
3.5	2.260	2.326	2.551	2.892
4.0	2.599	2.995	2.673	2.480
4.5	2.876	2.757	2.278	2.378
5.0	2.486	2.503	2.247	2.662
5.5	2.486	2.565	2.398	3.001
6.0	2.463	2.697	2.826	2.619

Table 6.17 Dry matter content of the whole body - treatment group means

	- phytase		+ phytase	
	female	male	female	male
Phosphorus level[r]	*% dry matter of the whole body*			
2.0	33.93	28.87	34.22	31.50
2.5	34.35	30.45	33.17	34.71
3.0	31.79	30.14	33.15	31.26
3.5	29.42	31.19	31.98	34.92
4.0	32.92	34.06	35.08	27.62
4.5	37.52	34.13	30.72	31.50
5.0	33.44	34.15	30.42	26.65
5.5	34.61	32.77	30.53	35.15
6.0	32.19	34.59	35.11	34.72

[r] non-phytate phosphorus g/kg freshweight diet

Figure 6.8 **Response of percent phosphorus in the dry matter of the body (y) to intake of phosphorus (x)**



Response equations

Linear	$y = 1.47 + 0.02x$	$p = 0.055$	% var = 7.7
Quadratic	$y = 1.32 + 0.12x - 0.01x^2$	$p = 0.056$	% var = 10.9

Table 6.18 Regression of phosphorus and ash content of the body (y) against daily non-phytate phosphorus intake (x)

Variate	*Linear*			*Quadratic*		
	Equation of response	P	% var[τ]	Equation of response	P	% var[τ]
Total phosphorus	$y = 103.6 + 6.87x$	<0.001	30.2	$y = 82.2 + 20.8x - 1.97x^2$	<0.001	31.6
DMP%	$y = 1.47 + 0.02x$	0.055	7.7	$y = 1.32 + 0.12x - 0.01x^2$	0.056	10.9
FWP%	$y = 0.44 + 0.02x$	0.007	17.1	$y = 0.35 + 0.08x - 0.01x2$	0.008	21.0
Total ash	$y = 490.6 + 38.20x$	<0.001	26.0	$y = 311.0 + 155.1x - 16.62x^2$	<0.001	30.8
DMash%	$y = 6.98 + 0.17x$	0.087	5.7	$y = 5.31 + 1.26x - 0.16x^2$	0.044	12.2
FWash%	$y = 2.10 + 0.11x$	0.006	17.5	$y = 1.31 + 0.62x - 0.07x^2$	0.002	26.9

[τ] % variance accounted for

Table 6.19 Regression of phosphorus and ash content of the body (y) against daily total phosphorus intake (x)

Variate	Linear			Quadratic		
	Equation of response	P	% var[τ]	Equation of response	P	% var[τ]
Total phosphorus	$y = 96.22 + 5.36x$	<0.001	31.9	$y = 65 + 16.9x - 0.98x^2$	0.001	32.6
DMP%	$y = 1.45 + 0.02x$	0.055	8.0	$y = 1.23 + 0.10x - 0.01x^2$	0.070	9.7
FWP%	$y = 0.43 + 0.01x$	0.008	16.6	$y = 0.23 + 0.07x - 0.01x^2$	0.010	19.6
Total ash	$y = 452.2 + 29.37x$	<0.001	26.5	$y = 189 + 126.7x - 8.30x^2$	<0.001	29.9
DMash%	$y = 6.84 + 0.13x$	0.096	5.2	$y = 4.41 + 1.02x - 0.08x^2$	0.072	9.6
FWash%	$y= 2.01 + 0.08x$	0.008	16.5	$y = 0.81 + 0.52x - 0.04x^2$	0.004	23.8

[τ] % variance accounted for

6.3.4 Physical properties of bone

The effects of phosphorus level, phytase, gender and the interactions on the physical properties of the femur and the third and fourth metatarsals are shown in table 6.20. Addition of phosphorus or phytase to the diet resulted in significant increases in femur strength ($p < 0.001$); the enzyme resulting in an increased femur strength at all levels of dietary phosphorus ($p < 0.001$, figure 6.9). In general, boars had heavier femurs than gilts, but this was not reflected in an increased strength.

No effects of phosphorus level on the physical characteristics of the third or fourth metatarsals were observed. Addition of phytase to the diet increased the strength of the fourth metatarsal, but not the third. Gender effects were apparent, with females having shorter and stronger metatarsals, although weight of these did not differ significantly between the sexes. Strength of the third metatarsal strength was more responsive to phytase in boars than in gilts. Data sets showing treatment group means are presented in tables 6.21 to 6.29.

Increasing daily intake of non-phytate phosphorus resulted in an increased femur strength. Linear and quadratic response curves of femur strength against daily non-phytate phosphorus intake are shown in figure 6.10. The responses were destroyed by the addition of phytase which altered the levels of non-phytate phosphorus. Equations describing the linear and quadratic responses of femur physical measurements (freshweight, length and strength) to daily intake of non-phytate phosphorus and daily intake of total phosphorus are presented in table 6.30.

Linear and quadratic relationships between increasing daily intake of phosphorus and the physical properties of the metatarsals were not apparent. There was a slight linear relationship between third metatarsal strength and daily intake of non-phytate phosphorus ($p=0.095$), and between third metatarsal strength and daily intake of total phosphorus ($p= 0.085$). These are given in table 6.31.

Table 6.20 **Significance of the effects of phosphorus level, phytase, sex and the interactions on physical properties of bones**

Variate	Factor	p	sed
Femur			
Freshweight	sex	<0.001	2.87
Length	ns	>0.10	-
Strength	P level	<0.001	8.32
	phytase	<0.001	3.85
3rd Metatarsal			
Freshweight	ns	>0.10	-
Length	sex	<0.001	1.63
	enzyme x sex	0.094	0.758
Strength	sex	0.071	2.34
	enzyme x sex	0.061	3.35
4th Metatarsal			
Freshweight	ns	>0.10	-
Length	sex	0.002	0.555
Strength	sex	0.024	0.565

Figure 6.9 Effect of phosphorus level and phytase on femur breaking strength

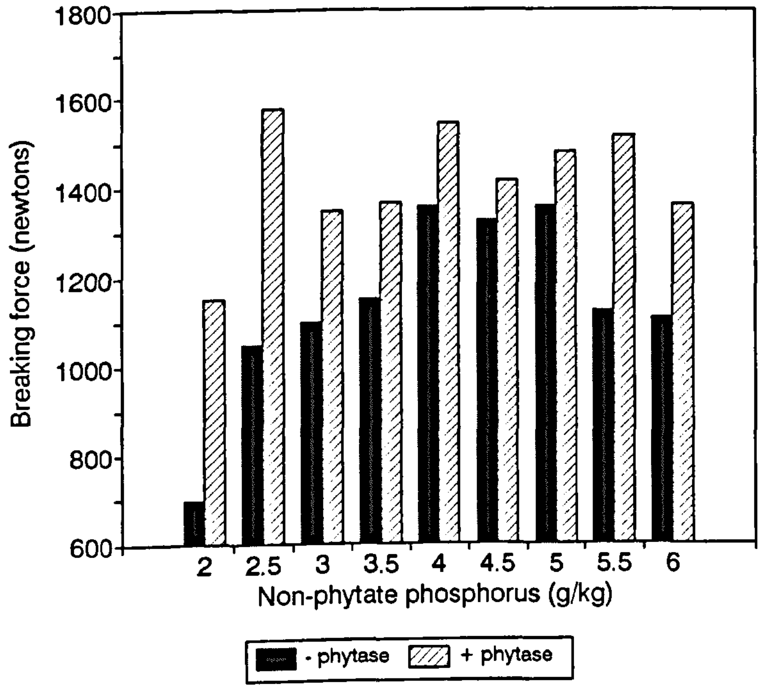


Table 6.21 Mean femur freshweight (g) of the treatment groups

P level	- phytase		+ phytase	
	female	male	female	male
2.0	117.2	111.5	117.7	129.5
2.5	114.2	112.2	128.6	123.0
3.0	102.3	123.1	118.1	116.0
3.5	114.9	117.2	121.3	128.7
4.0	100.8	128.1	112.0	123.9
4.5	115.3	122.7	113.3	124.1
5.0	107.1	127.1	93.5	121.1
5.5	114.6	128.7	97.4	136.9
6.0	115.8	119.2	122.9	130.5

Table 6.22 Mean femur length (mm) of the treatment groups

P level	- phytase		+ phytase	
	female	male	female	male
2.0	133.7	132.9	131.8	132.5
2.5	134.0	130.1	130.3	129.0
3.0	126.6	134.6	134.1	133.0
3.5	133.3	132.1	128.2	135.8
4.0	128.3	133.4	126.2	133.3
4.5	131.3	133.3	132.9	134.5
5.0	132.6	130.5	123.3	136
5.5	129.4	131.8	127.2	135
6.0	131.6	130.6	135.9	130.8

τ non-phytate phosphorus g/kg diet

Table 6.23 Mean femur strength (newtons) of the treatment groups

P level	- phytase		+ phytase	
	female	male	female	male
2.0	570	823	1122	1181
2.5	1158	933	1399	1753
3.0	976	1218	1305	1388
3.5	1221	1078	1453	1278
4.0	1398	1313	1271	1817
4.5	1342	1306	1358	1474
5.0	1430	1287	1475	148
5.5	1102	1145	1529	1498
6.0	1064	1147	1416	1304

Table 6.24 Mean third metatarsal freshweight (g) of the treatment groups

	- phytase		+ phytase	
P level	female	male	female	male
2.0	12.94	12.22	13.40	16.73
2.5	12.64	14.95	13.34	11.83
3.0	13.85	14.47	13.34	13.99
3.5	14.75	12.97	13.94	12.55
4.0	12.24	12.45	11.97	10.28
4.5	13.83	13.61	17.00	13.82
5.0	13.08	12.44	15.29	12.75
5.5	13.64	14.28	13.58	13.61
6.0	12.73	11.53	14.07	13.13

[τ] non-phytate phosphorus g/kg diet

Table 6.25 Mean third metatarsal length of the treatment groups

P level	- phytase		+ phytase	
	female	male	female	male
2.0	58.8	59.7	57.8	60.5
2.5	63.6	61.3	57.1	61.1
3.0	59.5	63.0	59.9	57.9
3.5	56.6	60.1	56.8	60.9
4.0	58.9	63.5	56.4	60.2
4.5	57.9	60.4	55.9	60.2
5.0	59.2	57.4	56.1	61.4
5.5	61.1	59.3	58.3	61.3
6.0	58.1	59.3	60.1	62.2

Table 6.26 Mean third metatarsal breaking strength (newtons) of the treatment groups

P level	- phytase		+ phytase	
	female	male	female	male
2.0	440	267	461	384
2.5	307	319	443	400
3.0	418	256	325	439
3.5	453	347	287	359
4.0	539	286	414	443
4.5	476	340	369	544
5.0	403	382	498	386
5.5	428	415	422	446
6.0	415	453	532	378

ᵀ non-phytate phosphorus g/kg diet

Table 6.27 Mean fourth metatarsal freshweight (g) of the treatment groups

P level	- phytase		+ phytase	
	female	male	female	male
2.0	15.21	13.42	12.20	14.77
2.5	14.45	16.20	13.73	14.17
3.0	16.37	14.38	20.70	14.43
3.5	14.05	14.87	14.55	17.18
4.0	13.76	18.60	13.55	14.57
4.5	12.66	13.35	13.65	13.73
5.0	15.51	15.89	13.43	16.81
5.5	11.89	14.11	13.18	13.80
6.0	15.16	15.62	12.47	15.02

Table 6.28 Mean fourth metatarsal length (mm) of the treatment groups

P level	- phytase		+ phytase	
	female	male	female	male
2.0	62.7	62.8	60.0	62.5
2.5	62.9	60.8	59.0	62.3
3.0	61.4	65.1	62.0	60.9
3.5	59.7	59.9	58.1	65.7
4.0	62.34	66.2	58.6	62.8
4.5	69.9	61.7	57.6	60.5
5.0	62.1	61.4	58.0	62.2
5.5	61.0	61.1	59.4	63.0
6.0	60.6	62.2	61.6	59.3

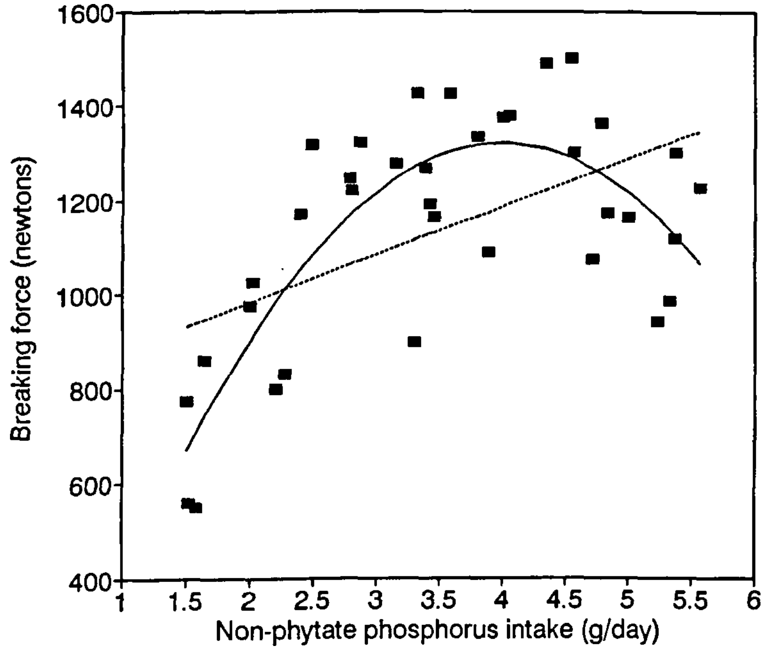
' non-phytate phosphorus g/kg diet

Table 6.29 Mean fourth metatarsal breaking strength (newtons) of the treatment groups

P level	- phytase		+ phytase	
	female	male	female	male
2.0	364	253	326	299
2.5	253	227	329	329
3.0	312	426	351	320
3.5	357	285	356	336
4.0	391	251	499	317
4.5	374	323	359	279
5.0	342	319	557	331
5.5	373	262	301	335
6.0	240	389	368	332

[τ] non-phytate phosphorus g/kg diet

Figure 6.10 Response of femur breaking strength (y) to phosphorus intake (x)



Response equations

Linear	$y = 77.7 + 10.23x$	$p = 0.001$	% var = 24.6
Quadratic	$y = -35.3 + 83.6x - 10.44x^2$	$p < 0.001$	% var = 59.7

Table 6.30 Regression of femur physical properties (y) against daily phosphorus intake (x)

	Linear			*Quadratic*		
	p	% var[τ]	response equation	p	% var[τ]	response equation
Daily non-phytate P intake						
Freshweight	0.057	7.6	$y = 103.97 + 3.41x$	ns	-	-
Length	ns	-	-	ns	-	-
Strength	0.001	24.6	$y = 77.7 + 10.23x$	<0.001	59.7	$y = -35.3 + 83.6x - 10.44x^2$
Daily total P intake						
Freshweight	0.051	8.1	$y = 100.33 + 2.65x$	ns	-	-
Length	ns	-	-	ns	-	-
Strength	<0.001	26.2	$y = 66.6 + 8.01x$	<0.001	59.3	$y = -115.4 + 75.2x - 5.73x^2$

Table 6.31 Linear response of third metatarsal strength (y) to daily phosphorus intake (x)

	p	% var[τ]	equation of response
Daily non-phytate P intake	0.095	5.7	$y = 29.65 + 2.62x$
Daily total P intake	0.085	6.2	$y = 26.87 + 2.04x$

[τ] percentage variance accounted for

6.3.5 Bone Mineralization

6.3.5.1 Femur

The effect of phosphorus level, phytase, gender and the interactions on mineral content of the fat free femur are given in table 6.32. Treatment group means are presented in tables 6.33 to 6.39. Total phosphorus in the femur was increased by addition of phytase ($p=0.013$; figure 6.11), although percent of phosphorus in the femur was not affected. Addition of phytase increased the fat free weight and ash content of the femur. Neither phosphorus level or phytase influenced the amount or percentage of calcium in the femur. Boars had heavier femurs, but gilts had a greater percentage of ash, although total ash did not differ between sexes.

A strong quadratic relationship between percent phosphorus in the femur and daily intake of non-phytate phosphorus ($p= 0.003$), and a weaker linear relationship ($p=0.03$) was apparent, as illustrated in figure 6.12. There were strong linear and quadratic relationships between daily non-phytate phosphorus intake and total ash, percent ash, and total phosphorus of the fat free femur. These are described in table 6.40. Equations describing linear and quadratic relationships between daily total phosphorus intake and femur mineral content are presented in table 6.41.

Table 6.32 Significance of the effects of phosphorus level, phytase, sex and the interactions on mineral content of the femur

Variate	Factor	p	sed
Fat free weight	P level	0.043	3.942
	Phytase	0.012	1.823
	Sex	0.002	1.826
	P level * phytase	0.088	5.584
Total ash	P level	0.002	0.792
	Phytase	<0.001	0.367
	P level * phytase	0.091	1.124
% ash	P level	0.016	1.053
	Phytase	0.021	0.487
	Sex	0.004	0.488
	P level * phytase	0.080	1.491
Total phosphorus	Phytase	0.013	0.150
% phosphorus	ns	-	-
Total calcium	ns	-	-
% calcium	ns	-	-

Table 6.33 Fat free weight of the femur - treatment group means

	- phytase		+ phytase	
	femal	male	female	male
Phosphorus level[r]	*fat free weight of the femur (g)*			
2.0	75.00	75.70	74.69	94.73
2.5	77.96	81.63	92.53	81.42
3.0	82.64	90.38	86.21	74.65
3.5	77.26	80.34	84.63	85.29
4.0	75.68	85.35	78.07	81.90
4.5	77.57	85.75	79.88	90.01
5.0	90.02	78.59	75.95	130.71
5.5	84.32	83.00	77.92	86.59
6.0	72.88	81.58	79.55	87.68

[r] non-phytate phosphorus g/kg freshweight diet

Table 6.34 Total ash in the femur - treatment group means

	- phytase		+ phytase	
	female	male	female	male
Phosphorus level[r]	total ash in the femur (g)			
2.0	11.82	12.72	16.72	16.52
2.5	15.36	12.97	19.54	17.72
3.0	15.37	15.80	17.93	16.07
3.5	17.32	15.50	17.58	16.88
4.0	17.42	17.05	17.50	17.89
4.5	17.89	17.74	16.90	19.00
5.0	18.72	16.15	17.97	19.56
5.5	16.75	16.04	17.21	19.25
6.0	15.94	17.68	17.64	17.76

Table 6.35 Percent ash in the femur - treatment group means

	- phytase		+ phytase	
	female	male	female	male
Phosphorus level[r]	% ash in the femur			
2.0	15.42	16.71	22.47	17.42
2.5	19.67	15.44	20.15	21.78
3.0	18.53	17.82	20.93	21.57
3.5	22.59	19.24	20.75	19.79
4.0	23.14	19.81	22.50	21.82
4.5	23.05	20.66	21.10	21.14
5.0	20.75	20.81	23.70	15.52
5.5	20.02	19.38	22.06	22.56
6.0	21.83	21.65	22.16	20.19

[r] non-phytate phosphorus g/kg freshweight diet

Table 6.36 Total phosphorus in the femur - treatment group means

	- phytase		+ phytase	
	female	male	female	male
Phosphorus level[r]		*total phosphorus in the femur (g)*		
2.0	2.30	2.42	3.29	3.478
2.5	3.28	2.81	4.47	3.49
3.0	2.63	2.89	3.61	3.01
3.5	3.78	3.17	3.60	3.57
4.0	3.95	3.86	3.29	4.07
4.5	2.96	3.57	3.67	3.27
5.0	3.57	3.24	3.63	4.16
5.5	3.32	3.58	3.18	4.15
6.0	3.37	2.74	3.74	2.83

Table 6.37 Percent phosphorus in the femur - treatment group means

	- phytase		+ phytase	
	female	male	female	male
Phosphorus level[r]		*% phosphorus in the femur*		
2.0	2.94	3.18	4.49	3.67
2.5	4.19	3.32	4.08	4.27
3.0	3.15	3.30	4.26	4.03
3.5	4.90	3.93	4.28	4.21
4.0	5.25	4.49	4.24	4.96
4.5	3.85	4.17	4.59	3.67
5.0	3.98	4.19	4.83	3.31
5.5	4.04	4.31	4.09	4.91
6.0	4.60	3.35	4.68	3.21

[r] non-phytate phosphorus g/kg freshweight of diet

Table 6.38 Total calcium in the femur - treatment group means

	- phytase		+ phytase	
	female	male	female	male
Phosphorus level[r]		*total calcium in the femur (g)*		
2.0	4.55	6.17	8.88	9.60
2.5	8.03	5.53	9.14	8.42
3.0	7.24	10.25	7.99	7.73
3.5	9.46	7.11	9.10	4.76
4.0	8.48	8.89	8.36	10.10
4.5	9.88	8.06	7.88	11.07
5.0	10.33	7.74	9.95	8.36
5.5	9.34	7.97	6.32	11.11
6.0	8.14	4.92	7.53	8.20

Table 6.39 Percent calcium in the femur - treatment group means

	- phytase		+ phytase	
	female	male	female	male
Phosphorus level[r]		*% calcium in the femur*		
2.0	5.78	8.14	12.02	10.17
2.5	10.49	6.53	9.96	10.34
3.0	8.71	11.46	9.31	10.18
3.5	12.24	8.83	10.77	5.84
4.0	11.50	10.28	10.72	12.62
4.5	12.73	9.79	9.95	12.26
5.0	11.47	9.96	13.10	6.77
5.5	11.16	9.47	8.16	12.95
6.0	11.08	6.03	9.66	9.29

[r] non-phytate phosphorus g/kg freshweight diet

Figure 6.11 Effect of phosphorus level and phytase on total phosphorus in the femur

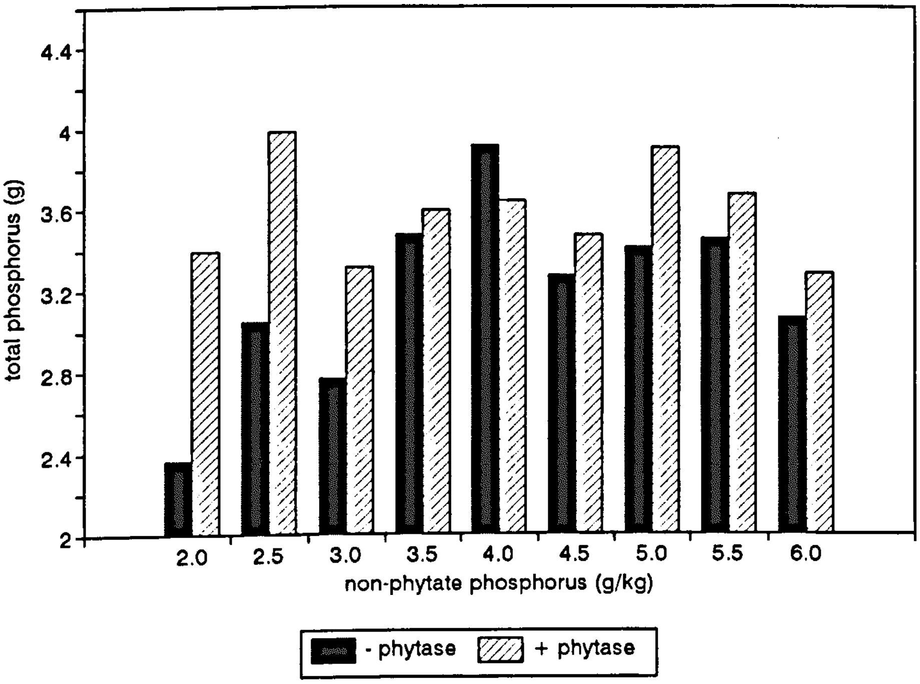
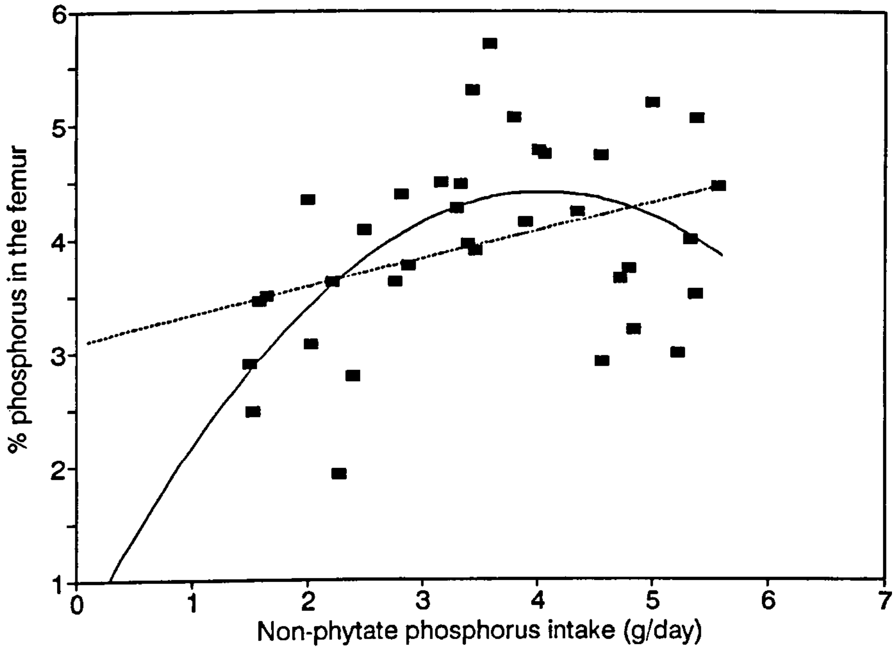


Figure 6.12 Response of percent phosphorus in the femur (y) to phosphorus intake (x)



Response equations

Linear	$y = 3.081 + 0.247x$	$p = 0.03$	% var = 10.6
Quadratic	$y = 0.45 + 1.95x - 0.24x^2$	$p = 0.003$	% var = 25.0

Table 6.40 Regression of femur mineral content (y) against daily non-phytate phosphorus intake (x)

	Linear			*Quadratic*		
Variate	Equation of response	p	% var[τ]	Equation of response	p	% var[τ]
Total ash in FFW	$y = 10.80 + 1.46x$	<0.001	50.6	$y = 4.01 + 5.88x - 0.63x^2$	<0.001	61.9
Percent ash in FFW	$y = 15.21 + 1.30x$	<0.001	35.9	$y = 7.40 + 6.37x - 0.72x^2$	<0.001	49.5
Total phosphorus in FFW	$y = 2.19 + 0.28x$	0.002	22.0	$y = 0.27 + 1.69x - 0.20x^2$	<0.001	36.2
Percent phosphorus in FFW	$y = 3.081 + 0.247x$	0.030	10.6	$y = 0.45 + 1.95x - 0.24x^2$	0.003	25.0
Total calcium in FFW	$y = 6.95 + 0.065x$	0.019	16.6	-	ns	-
Percent calcium in FFW	$y = 9.178 + 0.044x$	0.046	12.0	-	ns	-

[τ] % variance accounted for

Table 6.41 Regression of femur mineral content (y) against daily total phosphorus intake (x)

	Linear			*Quadratic*		
Variate	Equation of response	p	% var	Equation of response	p	% var
Fat free weight (FFW)	$y = 69.63 + 1.88x$	0.036	9.8	-	ns	-
Total ash in FFW	$y = 9.21 + 1.14x$	<0.001	53.6	$y = -1.37 + 5.05x - 0.33x^2$	<0.001	63.5
Percent ash in FFW	$y = 13.86 + 1.01x$	<0.001	37.3	$y = 1.17 + 5.69x - 0.401 x^2$	<0.001	50.3
Total phosphorus in FFW	$y = 1.85 + 0.22x$	0.001	24.8	$y = -1.7 + 1.537x - 0.11x^2$	<0.001	38.8
Percent phosphorus in FFW	$y = 2.79 + 0.20x$	0.022	12.0	$y = -1.65 + 1.84x - 0.14x^2$	0.002	8.0
Total calcium in FFW	$y = 6.60 + 0.035x$	0.019	16.7	-	ns	-
Percent calcium in FFW	$y = 8.86 + 0.025x$	0.051	11.5	-	ns	-

' % variance accounted for

6.3.5.2 Metatarsals

The effect of phosphorus level, phytase, sex and the interactions on fat free weight (FFW), total phosphorus, percent of phosphorus (%P), total calcium, percent of calcium (%Ca), total ash and percent of ash (%ash) in the fat free weight of the third and fourth metatarsals are given in table 6.42.

Addition of phytase to the diet resulted in an increased phosphorus weight of the fourth metatarsal ($p= 0.003$). There was an interacting phosphorus level x phytase x gender effect on the percent of phosphorus in the third metatarsal ($p= 0.051$) and fourth metatarsal ($p= 0.015$). Addition of phosphorus or phytase to the diet resulted in an increased ash content of the fourth, but not the third metatarsal.

Treatment means are presented in tables 6.43 - 6.56. Results suggested that the mineral content of the fourth metatarsal was sensitive to phosphorus level, but the third metatarsal, being less responsive, was not suitable as an indicator of phosphorus availability.

Strong linear ($p=0.002$) and quadratic ($p<0.001$) relationships between phosphorus in the fourth metatarsal and daily non-phytate phosphorus intake were observed (figure 6.13); and similarly between phosphorus in the fourth metatarsal and daily total phosphorus intake. Responses of fat free weight and ash of the fourth metatarsal to daily phosphorus intake were also seen. These are described in tables 6.57-6.60.

Table 6.42 **Significance of the effects of phosphorus level, phytase and sex on mineral content of the metatarsal**

Variate	Factor	p	sed
3rd Metatarsals			
Fat free weight	sex	0.058	0.411
Total ash	ns	-	-
% ash	ns	-	-
Total phosphorus	ns	-	-
% phosphorus	p level * phytase * sex	0.051	1.529
Total calcium	ns	-	-
% calcium	ns	-	-
4th metatarsals			
Fat free weight	P level	0.089	0.747
	P level * sex	0.054	1.048
Total ash	P level	0.011	0.102
	phytase	0.003	0.047
	P level * phytase	0.060	0.145
	sex	0.081	0.047
% ash	P level	0.063	3.750
Total phosphorus	phytase	0.003	0.018
% phosphorus	P level * phytase * sex	0.015	1.688
Total calcium	ns	-	-
% calcium	ns	-	-

Table 6.43 Fat free weight of the third metatarsal - treatment group means

	- phytase		+ phytase	
	female	male	female	male
Phosphorus level[r]	*fat free weight of MT3 (g)*			
2.0	6.68	7.53	7.15	9.15
2.5	6.87	8.99	8.54	8.83
3.0	7.16	9.71	8.22	7.00
3.5	7.80	6.07	7.30	10.15
4.0	6.89	8.53	6.36	8.27
4.5	7.43	8.52	8.38	7.59
5.0	7.63	10.86	7.91	8.71
5.5	8.05	8.25	6.72	8.08
6.0	11.79	7.42	6.31	8.02

Table 6.44 Fat free weight of the fourth metatarsal - treatment group means

	- phytase		+ phytase	
	female	male	female	male
Phosphorus level[r]	*fat free weight of MT4 (g)*			
2.0	4.25	7.47	4.85	8.17
2.5	4.23	6.46	6.69	7.83
3.0	4.94	6.29	6.82	4.46
3.5	8.73	5.72	7.75	6.62
4.0	7.75	4.67	5.93	8.36
4.5	6.96	7.76	6.36	6.03
5.0	6.30	7.27	5.72	6.74
5.5	7.37	8.27	9.71	7.84
6.0	6.38	7.47	6.91	7.19

[r] non-phytate phosphorus g/kg freshweight diet

Table 6.45 Total ash in the third metatarsal - treatment group means

	- phytase		+ phytase	
	female	male	female	male
Phosphorus level[r]		*total ash in fat free MT3 (g)*		
2.0	1.20	1.57	1.86	1.87
2.5	1.22	1.42	2.59	2.20
3.0	1.89	3.43	2.34	1.77
3.5	2.01	1.73	1.71	2.94
4.0	2.15	1.88	1.93	2.04
4.5	1.96	1.79	1.82	2.22
5.0	1.77	3.94	2.09	2.46
5.5	2.11	2.05	1.69	2.27
6.0	2.02	2.23	1.91	1.20

Table 6.46 Percent ash in the third metatarsal - treatment group means

	- phytase		+ phytase	
	female	male	female	male
Phosphorus level[r]		*% ash in fat free MT3*		
2.0	18.79	20.98	26.46	20.60
2.5	16.98	15.72	29.79	24.99
3.0	26.39	33.97	30.66	28.81
3.5	25.60	29.39	24.59	29.53
4.0	31.17	22.43	30.29	24.45
4.5	26.37	21.28	22.96	28.78
5.0	23.60	34.81	25.99	27.96
5.5	25.69	24.60	25.24	27.60
6.0	21.21	30.09	30.67	24.89

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[r] non-phytate phosphorus g/kg freshweight diet

Table 6.47 Total ash in the fourth metatarsal - treatment group means

	- phytase		+ phytase	
	female	male	female	male
Phosphorus level[r]		*Total ash in fat free MT4 (g)*		
2.0	1.28	1.53	1.74	1.89
2.5	1.75	1.49	1.92	2.17
3.0	1.83	1.87	2.09	1.79
3.5	1.76	1.72	1.83	2.08
4.0	2.03	1.99	1.90	1.95
4.5	1.93	1.88	1.79	2.21
5.0	1.98	1.91	2.01	2.18
5.5	1.80	1.70	1.95	2.05
6.0	1.77	2.44	1.88	1.90

Table 6.48 Percent ash in the fourth metatarsal - treatment group means

	- phytase		+ phytase	
	female	male	female	male
Phosphorus level[r]		*% ash in fat free MT4*		
2.0	32.18	20.68	36.91	23.47
2.5	41.93	23.95	28.28	27.85
3.0	37.72	29.75	33.10	40.23
3.5	19.66	30.70	24.17	33.60
4.0	26.19	42.82	32.20	23.22
4.5	27.69	25.08	29.03	36.88
5.0	33.50	31.85	35.49	34.78
5.5	23.72	20.15	19.24	25.76
6.0	27.96	32.63	27.36	27.28

[r] non-phytate phosphorus g/ kg freshweight diet

Table 6.49 Total phosphorus in the third metatarsal - treatment group means

	- phytase		+ phytase	
	female	male	female	male
Phosphorus level[r]	*Total phosphorus in fat free MT3 (g)*			
2.0	0.25	0.28	0.12	0.45
2.5	0.33	0.18	0.24	0.32
3.0	0.36	0.22	0.33	0.29
3.5	0.31	0.28	0.34	0.36
4.0	0.46	0.41	0.25	0.31
4.5	0.22	0.33	0.35	0.25
5.0	0.33	0.26	0.24	0.36
5.5	0.33	0.24	0.48	0.46
6.0	0.24	0.39	0.43	0.29

Table 6.50 Percent phosphorus in the third metatarsal - treatment group means

	- phytase		+ phytase	
	female	male	female	male
Phosphorus level[r]	*% phosphorus in fat free MT3*			
2.0	3.77	3.74	2.83	5.51
2.5	5.24	1.90	3.27	3.73
3.0	5.03	2.33	4.26	5.07
3.5	3.91	4.62	4.72	3.60
4.0	6.72	4.95	3.93	3.69
4.5	3.01	3.90	4.51	3.23
5.0	4.43	2.10	3.03	4.23
5.5	4.12	2.91	5.12	5.55
6.0	2.47	5.08	6.92	3.62

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[r] non-phytate phosphorus g/kg freshweight diet

Table 6.51 Total phosphorus in the fourth metatarsal - treatment group means

	- phytase		+ phytase	
	female	male	female	male
Phosphorus level[r]	*Total phosphorus in fat free MT4 (g)*			
2.0	0.21	0.24	0.37	0.43
2.5	0.38	0.27	0.36	0.47
3.0	0.40	0.36	0.42	0.39
3.5	0.34	0.38	0.40	0.50
4.0	0.29	0.40	0.42	0.43
4.5	0.38	0.40	0.39	0.38
5.0	0.43	0.43	0.42	0.43
5.5	0.39	0.34	0.42	0.46
6.0	0.34	0.44	0.40	0.38

Table 6.52 Percent of phosphorus in the fourth metatarsal - treatment group means

	- phytase		+ phytase	
	female	male	female	male
Phosphorus level[r]	*% phosphorus in fat free MT4 (g)*			
2.0	5.38	3.30	7.68	5.29
2.5	9.20	4.29	5.23	6.07
3.0	8.34	5.31	6.58	8.74
3.5	3.76	6.18	5.39	7.89
4.0	3.55	9.79	7.09	5.04
4.5	5.51	5.39	6.43	6.34
5.0	7.25	4.01	7.35	7.03
5.5	4.99	3.97	3.98	5.67
6.0	5.28	6.00	5.85	5.46

[r] non-phytate phosphorus g/kg freshweight diet

Table 6.53 Total calcium in the third metatarsal - treatment group means

	- phytase		+ phytase	
	female	male	female	male
Phosphorus level[r]	*total calcium in fat-free MT3 (g)*			
2.0	0.34	0.63	0.59	1.09
2.5	1.05	0.41	0.86	0.95
3.0	0.96	1.01	0.69	1.16
3.5	1.19	0.63	0.80	0.89
4.0	0.82	0.74	0.76	1.03
4.5	0.79	0.75	0.67	0.68
5.0	0.78	1.02	1.27	1.25
5.5	0.65	0.63	0.72	0.95
6.0	1.27	1.24	0.97	1.14

Table 6.54 Percent calcium in the third metatarsal - treatment group means

	- phytase		+ phytase	
	female	male	female	male
Phosphorus level[r]	*% calcium in fat free MT3*			
2.0	6.13	8.66	6.92	13.59
2.5	17.01	5.85	11.23	11.51
3.0	13.29	9.42	13.27	12.97
3.5	9.98	10.53	12.21	9.01
4.0	11.70	9.60	11.72	12.21
4.5	10.25	9.10	8.53	9.00
5.0	10.38	11.32	15.85	14.14
5.5	8.55	6.96	13.39	13.39
6.0	13.21	15.18	15.70	15.70

[r] non-phytate phosphorus g/kg freshweight diet

Table 6.55 Total calcium in the fourth metatarsal - treatment group means

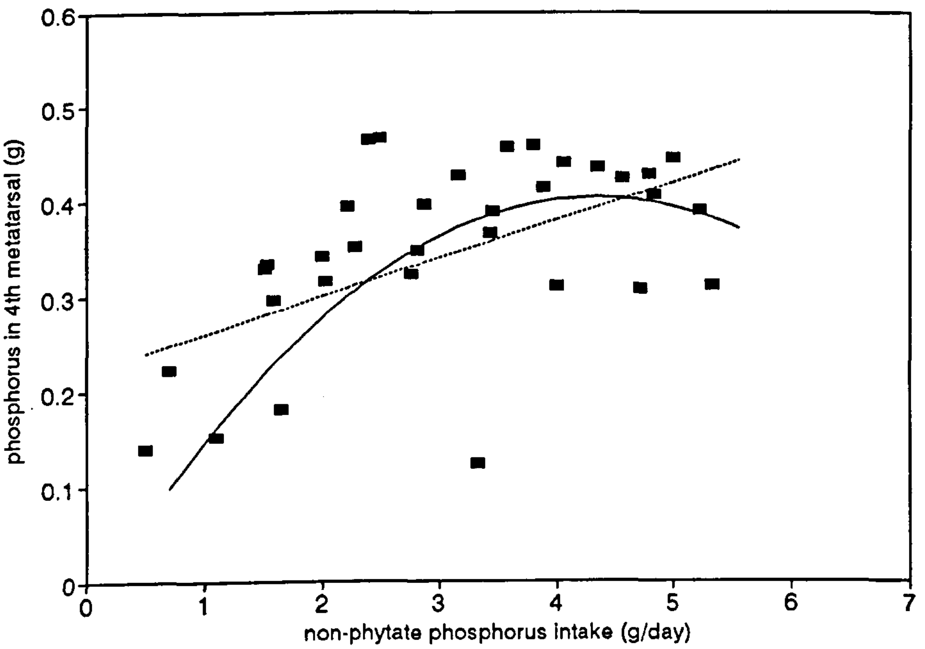
	- phytase		+ phytase	
	female	male	female	male
Phosphorus level[r]	*total calcium in fat-free MT4 (g)*			
2.0	0.26	0.07	0.07	0.11
2.5	0.17	0.26	0.15	0.09
3.0	0.33	0.10	0.11	0.20
3.5	0.11	0.67	0.15	0.10
4.0	0.36	0.16	0.06	0.05
4.5	0.11	0.20	0.38	0.36
5.0	0.53	0.15	0.30	0.32
5.5	0.15	0.26	0.19	0.30
6.0	0.20	0.10	0.13	0.21

Table 6.56 Percent calcium in the fourth metatarsal - treatment group means

	- phytase		+ phytase	
	female	male	female	male
Phosphorus level[r]	*% calcium in fat free MT4*			
2.0	5.07	0.93	1.43	1.39
2.5	3.70	4.59	3.56	1.15
3.0	7.27	1.83	1.95	3.98
3.5	1.87	13.06	2.04	1.27
4.0	5.07	3.85	0.76	0.68
4.5	1.59	3.04	6.82	3.97
5.0	6.86	2.01	3.26	6.90
5.5	2.50	2.87	1.89	3.41
6.0	3.21	1.55	1.99	0.68

[r] non-phytate phosphorus g/kg freshweight diet

Figure 6.13 **Response of phosphorus weight of the fourth metatarsal (y) to phosphorus intake (x)**



Response equations

Linear	$y = 0.22 + 0.04x$	$p = 0.002$	% var = 24.4
Quadratic	$y = -0.03 + 0.20x - 0.02x^2$	$p < 0.001$	% var = 33.9

Table 6.57 Regression of mineral content of the third metatarsal (y) against daily non-phytate phosphorus intake (x)

	Linear			*Quadratic*		
Variate	Equation of response	p	% var[r]	Equation of response	p	% var[r]
Fat free weight (FFW)	$y = 6.26 + 0.52x$	0.07	6.7	-	ns	-
Total ash in FFW	$y = 1.35 + 0.19x$	0.089	5.6	-	ns	-
Percent ash in FFW	-	ns	-	-	ns	-
Total phosphorus in FFW	-	ns	-	-	ns	-
Percent phosphorus in FFW	-	ns	-	-	ns	-
Total calcium in FFW	-	ns	-	-	ns	-
Percent calcium in FFW	-	ns	-	-	ns	-

[r] percentage variance accounted for

Table 6.58 Regression of mineral content of the third metatarsal (y) against daily intake of total phosphorus (x)

	Linear			Quadratic		
Variate	Equation of response	p	% var$^{\tau}$	Equation of response	p	% var$^{\tau}$
Fat free weight (FFW)	$y = 5.86 + 0.38x$	0.083	5.9	-	ns	-
Total ash in FFW	$y = 1.15 + 0.15x$	0.086	5.8	-	ns	-
Percent ash in FFW	-	ns	-	-	ns	-
Total phosphorus in FFW	-	ns	-	-	ns	-
Percent phosphorus in FFW	-	ns	-	-	ns	-
Total calcium in FFW	-	ns	-	-	ns	-
Percent calcium in FFW	-	ns	-	-	ns	-

$^{\tau}$ percentage variance accounted for

Table 6.59 Regression of mineral content of the fourth metatarsal (y) against daily intake of non-phytate phosphorus (x)

	Linear			Quadratic		
Variate	Equation of response	p	% var[τ]	Equation of response	p	% var[τ]
Fat free weight (FFW)	$y = 4.76 + 0.51x$	0.026	11.1	$y = 4.33 + 0.79x - 0.04x^2$	0.087	8.5
Total ash in FFW	$y = 1.29 + 0.15x$	<0.001	35.9	$y = 0.55 + 0.62x - 0.07x^2$	<0.001	44.6
Percent ash in FFW	-	ns	-	-	ns	-
Total phosphorus in FFW	$y = 0.22 + 0.04x$	0.002	24.4	$y = -0.03 + 0.20x - 0.02x^2$	<0.001	33.9
Percent phosphorus in FFW	-	ns	-	-	ns	-
Total calcium in FFW	-	ns	-	-	ns	-
Percent calcium in FFW	-	ns	-	-	ns	-

[τ] percentage variance accounted for

Table 6.60 Regression of mineral content of the fourth metatarsal (y) against daily intake of total phosphorus (x)

	Linear			Quadratic		
Variate	Equation of response	p	% var[τ]	Equation of response	p	% var[τ]
Fat free weight (FFW)	$y = 4.3 + 10.70x$	0.029	10.7	$y = 4.03 + 0.48x - 0.01x^2$	0.095	8.0
Total ash in FFW	$y = 1.14 + 0.11x$	<0.001	36.3	$y = -0.12 + 0.58x - 0.04x^2$	<0.001	50.3
Percent ash in FFW	-	ns	-	-	ns	-
Total phosphorus in FFW	$y = 0.18 + 0.03x$	0.002	25.1	$y = -0.25 + 0.19x - 0.01x^2$	<0.001	35.6
Percent phosphorus in FFW	-	ns	-	-	ns	-
Total calcium in FFW	-	ns	-	-	ns	-
Percent calcium in FFW	-	ns	-	-	ns	-

[τ] percentage variance accounted for

6.3.6 Relationship between femur strength and femur mineral content

Relationships between femur strength and mineral content are described in table 6.61. Strong linear and curvilinear relationships between femur strength and phosphorus, calcium and ash content of the femur ($p < 0.001$) were destroyed by phytase addition.

6.3.7 Relationship between femur mineral and whole body mineral content

Relationships between bone mineral and whole body mineral content are given in table 6.62. Strong linear relationships existed between ash in the femur and ash in the body. Conversely, those between phosphorus in the femur and the body were weak.

6.3.8 Relationship between femur strength and whole body mineral content

Relationships between femur strength and phosphorus, calcium and ash in the body are presented in table 6.63. A strong quadratic response of percent of ash in the dry matter of the carcase to femur strength was observed ($p = 0.006$). Relationships between femur strength and total ash, total phosphorus and percent phosphorus in the body were somewhat weaker (>0.01). Phytase addition destroyed all linear relationships except those between femur strength and percent ash in the carcase dry matter, and between femur strength and percent phosphorus in the carcase dry matter. Quadratic relationships were destroyed except for that between femur strength and percent phosphorus in the carcase dry matter.

6.3.9 Relationship between growth and whole body mineral content

Table 6.64 shows the total amount of phosphorus and ash required in the whole body to support growth, from which it could be seen that 4.28g of phosphorus was necessary for each kg of liveweight gain. Addition of phytase weakened the relationships between growth and mineral content of the body.

Table 6.61 **Regression of femur mineral content (y) against femur strength (x)**

	p	% var’	equation of response	p	% var’	equation of response
Linear				Quadratic		
Total ash	<0.001	45.5	$y = 7.2 + 6.60x$	<0.001	55.1	$y = -269.9 + 43.7x - 1.202x^2$
Ash %	<0.001	42.1	$y = - 8.6 + 6.18x$	<0.001	51.0	$y = -365 + 42.6x - 0.914x^2$
Total Phosphorus	<0.001	44.4	$y = 38.9 + 23.57x$	<0.001	46.3	$y = -30.4 + 71.4x - 7.81x^2$
Phosphorus %	<0.001	35.6	$y = 43.6 + 17.79x$	<0.001	35.0	$y = 0.4 + 41.5x - 3.06x^2$
Total calcium	<0.001	32.6	$y = 62.8 + 6.47x$	>0.10	-	ns
Calcium %	<0.001	28.0	$y = 64.41 + 5.08x$	<0.001	55.1	$y = -269.9 + 43.7x - 1.202x^2$

Table 6.62 **Regression of whole body mineral content (y) against femur mineral content (x)**

	P	% var^r	Equation of response	P	% var^r	Equation of response
	Linear				Quadratic	
Total ash in femur vs total ash in body	<0.001	31.4	$y = 297.6 + 20.54x$	>0.10	-	ns
% ash in femur vs DMash%	>0.10	-	ns	>0.10	-	ns
% ash in femur vs FWash%	0.009	16.1	$y = 1.498 + 0.050x$	>0.10	-	ns
Total phosphorus in femur vs total phosphorus in body	0.023	11.7	$y = 102.3 + 8.02x$	0.061	10.5	$y = -14.73 + 0.12x - 0.0004x^2$
% phosphorus in femur vs DMP%	>0.10	-	ns	>0.10	-	ns
% phosphorus in femur vs FWP%	>0.10	-	ns	>0.10	-	ns

Table 6.63 Regression of whole body mineral content (y) against femur strength (x)

	p	% var^r	Equation of response	p	% var^r	Equation of response
linear				*quadratic*		
Total ash	0.013	14.4	$y = 43.4 + 0.11x$	0.027	14.8	$y = 229 + 6.1x - 0.022^2$
DM ash%	0.011	14.9	$y = 7.0 + 14.1x$	0.006	22.4	$y = 2.78 + 0.81x - 0.0003x^2$
FW ash%	0.022	12.0	$y = 37.4 + 30.8x$	0.067	10.0	$y = 1.58 + 0.01x - 0.0004x^2$
Total phosphorus	0.058	7.4	$y = 47.7 + 0.52x$	ns	-	-
DMP%	0.058	7.5	$y = -30.4 + 93.2x$	0.072	9.5	$y = 1.15 + 0.01x = 0.0003x^2$
FWP%	ns	-	-	ns	-	-
Total calcium	ns	-	-	ns	-	-
DMCa%	ns	-	-	ns	-	-
FWCa%	ns	-	-	ns	-	-

^{r} percentage variance accounted for

Table 6.64 Regression of whole body mineral content (y) against daily liveweight gain (x)

	p	% var[τ]	equation of response	p	% var[τ]	equation of response
	- phytase			+ phytase		
Total ash	0.002	22.8	$y = 205 + 25.51x$	0.035	9.8	$y = 316 + 20.76$
Total phosphorus	0.002	22.8	$y = 57.3 + 4.28x$	0.066	6.9	$y = 71.3 + 3.72x$

[τ] percentage variance accounted for

6.4 Discussion

6.4.1 Growth performance

Linear and quadratic responses of growth and feed intake to daily phosphorus intake contrasted to those of Pond *et al.* (1975), Hines *et al.* (1979) and Nimmo *et al.*, (1980), where no effect of phosphorus intake on growth performance was observed. However, in these experiments, responses were measured at phosphorus levels above, rather than below requirement. In contrast, linear increases in digestibility and feed intake, and a quadratic response of feed conversion efficiency was found when available phosphorus was increased from 1.75 to 3.25g/kg (Combs *et al.*, 1991). Other work using pigs of 18-57kg showed that daily gain and feed intake increased linearly, but feed conversion efficiency was unaffected when regressed against total phosphorus levels of 4-9 g/kg (Maxson and Mahan, 1983).

These differences in growth responses demonstrate the importance of standardising conditions under which growth experiments are performed, if results are to be comparable. In the present experiment, growth parameters were regressed against daily phosphorus intake rather than dietary phosphorus level, because animals were receiving *ad libitum* feed and thus two animals receiving the same dietary level of phosphorus may have consumed markedly different amounts. In many of the experiments cited, growth performance was regressed against phosphorus level, even though the animals were fed *ad libitum*, and this may account for some of the differences in results. While it is generally assumed that growth performance is not a sensitive indicator of phosphorus requirement or availability, present results suggested that if regressed against phosphorus intake, rather than dietary level, growth performance was closely correlated with dietary digestible phosphorus.

Based on the quadratic equations of the present experiment, maximum daily gain (625g) was achieved at a daily intake of 5.25g non-phytate phosphorus ($y = 0.46 + 0.063x - 0.006x^2$); however, maximum feed intake (957g) required 6.67g non-phytate phosphorus ($y = 0.69 + 0.08x - 0.006x^2$). The ARC (1981) recommended requirements are somewhat lower than this, ranging from 2.7 g/day for 5kg pigs, to

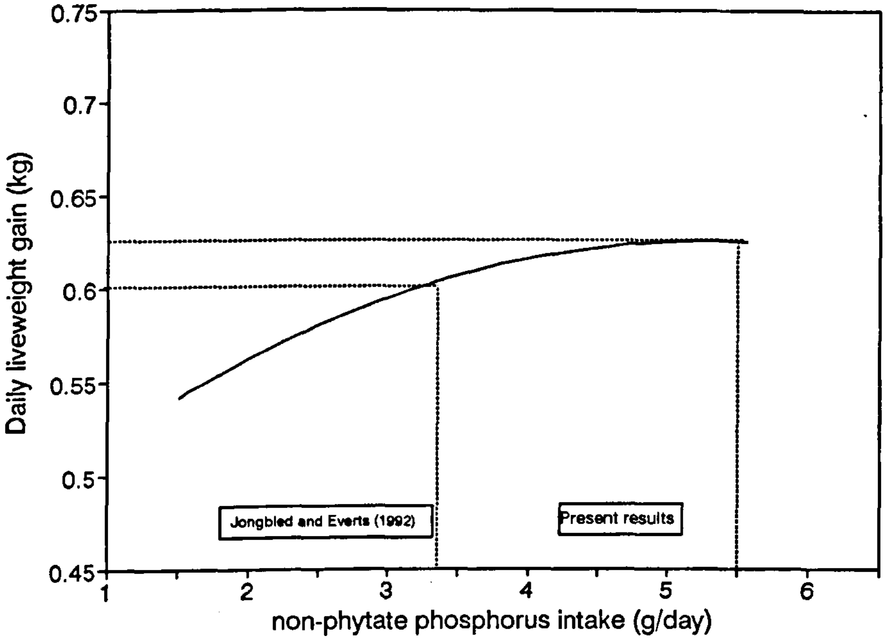
4.6 g/day for 25kg pigs. It was not possible from this experiment to calculate requirements at discreet liveweights, but it can be seen that the "average" requirement for 10-25kg pigs is somewhat greater than the "average" requirement that would be obtained using ARC recommendations.

Jongbloed and Everts (1992) recommended that 20kg pigs require 3.38g digestible phosphorus/day to achieve daily gains of 670g and daily feed intakes of 950g. From the present results, a daily intake of 3.38g digestible phosphorus would give a daily gain of only 604g, and a feed intake of 890 g/day (figure 6.14). In an experiment using pigs from 8-25kg liveweight, maximum daily gain was achieved at 0.3% phosphorus (Mori and Takada, 1990), which in light of present results seems extremely low. Based on the present mean daily feed intake of 1.48kg freshweight, a dietary concentration of 3.7 g digestible phosphorus would be required for pigs of 10-25kg. This was in agreement Chatelier (1989) who suggested that pigs of this size require 3.8g/kg available phosphorus (equivalent to 10mg/kg liveweight).

Lowered feed intake of pigs receiving the basal diet (which was markedly deficient in phosphorus) suggested that it was the primary causative factor of lowered daily gain. Weaned pigs fed calcium to phosphorus ratios from 0.8:1 to 3.2:1 showed a decrease in average daily gain which was not fully attributed to decreased voluntary feed intake (Weingand *et al.*, 1988), but in the present experiment feed conversion efficiency was largely unaffected.

Lowered average daily gains at the basal level of phosphorus were overcome by the addition of phytase. Again, this effect was largely due to increased feed intake, with little effect on feed conversion efficiency. Enzyme use is often associated with an increased voluntary feed intake, and this may have partially accounted for the increased feed intakes obtained, as slight increases were observed at most dietary phosphorus levels. However, the large response of pigs receiving the basal diet suggested that phytase stimulated appetite through increased phosphorus availability. Results were similar to those of Cromwell *et al.* (1993) where rate and efficiency of gain were decreased with phosphorus deficient diets, but these decreases were counteracted by addition of phytase.

Figure 6.14 Phosphorus requirement for growth; comparison of Jongbloed and Everts (1992) and present data



6.4.2 Carcase mineral content

Maximum amount of phosphorus in the whole body was achieved at a daily intake of 5.5g non-phytate phosphorus (based on the quadratic response). Although this corresponded to the intake required for maximum daily gain, concentration of phosphorus in the dry matter of the body also reached a maximum at an intake of 5.5g non-phytate phosphorus/day. Thus the greater amount of phosphorus in the body was not only due to increased body size.

Linear and curvilinear relationships between phosphorus intake and ash and phosphorus content of the body were similar in nature to results obtained by Ketaren *et al.* (1992) for growing pigs. These workers found that phosphorus and ash content of the whole body were the most responsive to phosphorus intake. In the present experiment, total phosphorus and total ash in the carcase appeared to be the most responsive to phosphorus intake, however, this may have been due simply to values of total mineral being much larger than values of percent mineral; thereby allowing greater sensitivity during the regression.

Variation in phosphorus content from 1.42 to 1.68 percent in the dry matter was observed (coefficient of variation = 5.5%). The mean fresh matter phosphorus content of 0.52% was similar to the value of 0.49% for pigs weighing 22kg obtained by Walz and Pallauf (1991). These workers found that phosphorus concentration declined with age; pigs weighing 5kg liveweight contained 0.54% phosphorus in the freshweight. Calcium content of the whole body, on the other hand, was extremely variable, ranging from 1.22 to 11.8 percent in the dry matter, and 0.41 to 3.48 percent in the freshweight (coefficient of variation 80%). Mean calcium to phosphorus ratio in the body was 3.83. Mean ash content of the freshweight was 2.54%; which was a little lower than the 3% predicted by Close (1994), for pigs of more than 5kg liveweight. The latter author suggested that ash content per unit weight of the carcase may serve as an index of mineralization of the animal as a whole, and from the present work, where ash content correlated closely to phosphorus content, this appeared to be true.

The maximum growth rate of 625g/day corresponded to 137g of phosphorus in the whole body. From the regression of growth against phosphorus in the whole body, it appeared that 4.28g of phosphorus was required for each kg of liveweight gain, which is lower than the estimate by Jongbloed (1987) of 5.4g P/kg liveweight gain in 10-20kg pigs. Interestingly, it appeared that in the presence of phytase only 3.72g of phosphorus was required for 1kg of liveweight gain, although whether this is valid given the weak relationship is unsure. Phytase would not be expected to give a lower requirement of available phosphorus for tissue growth.

Boars had a higher percentage of phosphorus and ash in the dry matter than gilts. This would be presumed to be due to a greater amount of bone, although other workers (Doornebal *et al.*, 1975; Shields, 1983 and Rook and Ellis, 1987) found no difference in the amount of bone between boars and gilts. Thus the higher phosphorus content in boars may have been due to more intensive mineralization of the skeleton. Calcium in the carcase was not influenced by dietary phosphorus, phytase or gender. In contrast, Schanler *et al.* (1991), investigating the distribution of calcium and phosphorus during various degrees of mineral deficiency, found that at intakes of 20% of the recommended phosphorus levels, both calcium and phosphorus content of the whole carcase was reduced.

Addition of phytase increased phosphorus content in the dry matter of the body at levels of up to 5.5g non-phytate phosphorus/kg, due to phosphorus liberated from phytate being assimilated into the skeleton and possibly also the soft tissues. Destruction of the strong linear and quadratic responses of mineral content in the body to phosphorus intake necessitated a re-evaluation of the methodology used. The experimental design was based on the assumption that at each level of dietary phosphorus, addition of phytase would release the same amount of phytate-phosphorus, so that a second regression could be fitted through the results of pigs receiving phytase. Response curves of pigs with and without the enzyme could then be compared. However, results suggested either that different amounts of phytate-phosphorus were being released at each level of dietary phosphorus, or that homeostatic regulation of phosphorus was influencing the amount of mineral in the carcase (or a combination of the two). The net effect of the latter would be a lesser effect of phytase as dietary phosphorus neared requirement. In the case of the former, a differential effect of phytase according to phosphorus content of the diet may have been due to an inhibiting effect of inorganic phosphorus on phytase activity. One exception was the weak relationship between phosphorus percent in the dry matter of the carcase and phosphorus intake, which retained its significance upon addition of phytase ($p=0.069$ with phytase, $p=0.056$ without phytase).

Mineral partitioning between the skeleton and soft tissues was not measured, but it is probable that the observed effects were partly due to changes in phosphorus content of the soft tissues, and thus both dietary phosphorus level and phytase may have important implications for carcase quality. Any effect of dietary phosphorus on mineral composition of the soft tissues may influence sensory properties of the meat. Furthermore, alteration of growth rates by dietary phosphorus may influence carcase composition of the slaughtered pig.

6.4.3 Physical properties of bones

Bone strength has been used by nutritionists to assess mineralization, and was concluded to be was the best trait from which to estimate the availability of dietary phosphorus (Hayes, 1979). The Instron tester is suitable for this purpose as it provides a constant load. The ideal bone for assessing mineralization varies with age and sex. Phosphorus requirements for maximum development differ widely, depending upon the bone used (Crenshaw *et al.*, 1981). Tanksley (1979) concluded that the femur was a better indicator of bone development than the metacarpal. Present results indicated that in young pigs the femur was more sensitive than either the metacarpal or the metatarsal.

The flexure test has been used extensively for determining mechanical properties of bones. The resultant curve corresponds to deformation of the bone. In the initial phase of the curve, force increased linearly and was proportional to deformation. During this phase elastic deformation occurred up to the inflection point, beyond which plastic deformation (permanent damage) took place (Evans, 1973). The test was stopped when the curve reached a peak, which corresponded to maximum force withstood by the bone before breaking.

Lack of standardisation during test procedures has resulted in variation in reported values for bone strength. Bones are irregular in shape, presenting problems for the determination of some physical properties. Although in many former experiments, stress, moment of inertia and modulus of elasticity have been calculated from bone breaking data (stress being defined as the force per unit area, moment of inertia as the area over which the force is being distributed, and modulus of elasticity as the rigidity of the bone), these calculations require measurement of the cross sectional area at the midshaft of the bone. As these bones were almost always splintered or broken after the break-strength measurements, it was impractical to measure the cross-sectional diameter, which would have required a "clean cut" surface at the midshaft. In this experiment, strength of the bone as a whole (ie the maximum force withstood before breaking), was the only trait used.

The bones used in this experiment were frozen in airtight bags immediately after being removed from the animal, and defrosted prior to testing. In this way they could be considered "fresh" or "wet", as opposed to "dry" bones which have undergone autoclaving and/or fat extraction. Differences exist in the properties of dry and wet bones. Wet bones tend to bend to a greater extent, but withstand less ultimate force than dried bones (Miller *et al.*, 1965). Wet bones resemble more closely the bones as they exist in the animal.Even a short period of exposure to air can result in changes in mechanical properties (Sedlin and Hirsch, 1966). Thus, throughout preparation for testing, bones were kept moist with 0.9% saline solution.

Increases in femur strength in response to increasing phosphorus intake were in accordance with results of Nimmo *et al.* (1980), Batterham *et al.* (1993) and Cromwell *et al.* (1993) who observed linear increases in response to increasing phosphorus intake. In the present experiment, both linear and curvilinear relationships were obtained and it is proposed that the linearity observed was in fact due to the "linear" (ie ascending) part of the quadratic curve. A curvilinear model would be a more rational model for bone development as it is unlikely that bone strength increases limitlessly with increasing phosphorus intake.

Based on the quadratic response, an average of 0.6g non-phytate phosphorus was necessary for each 10 newton increase in break strength. Maximum strength of the femur was achieved with a daily intake of 4g non-phytate phosphorus. Any further intake decreased bone strength, probably due to an incorrect calcium to phosphorus ratio for bone accretion. Boars had heavier femurs than gilts. Although the former are generally faster growing, no growth differences due to gender were found in the present experiment; thus it seems unlikely than the increase in strength would be due to an increased overall size of the bones. On the other hand, no difference in femur strength was detected between boars and gilts and thus it also seems unlikely that weight differences were due solely to increased mineralization.

Addition of phytase resulted in an increased femur strength at all levels of dietary phosphorus, which, based on previous results, could be attributed to an increased digestibility of phytate-phosphorus, and subsequent accretion of the free phosphates into skeletal tissue. The increase in femur strength was greater than that achieved by inorganic phosphate addition. In agreement with results of the whole body mineral analysis, addition of the enzyme acted to mask the regression responses to phosphorus intake, suggesting that homeostasis and/or interactions with inorganic phosphate were influencing the response to phytase. Although the response to phytase diminished as dietary phosphorus level increased, based on a comparison of femur strength with and without the enzyme at the basal phosphorus level, it was calculated that addition of phytase to the basal diet enabled approximately 70% of the phytate-phosphorus to be utilised (table 6.66).

The weak linear relationships that existed between metatarsal strength and phosphorus intake were evidence that these bones were unsuitable as indicators of phosphorus availability, and consequently were not responsive to phytase. The femur seemed to be a better indicator of phosphorus availability for pigs of this age, perhaps because of their earlier development.

6.4.4 Bone mineralization

Strong linear and quadratic relationships between total and percent ash in the femur, suggested that these were suitable as criteria for assessing mineralization. Strong quadratic relationships between percent of phosphorus in the femur and phosphorus intake also existed. Based on quadratic responses, maximum mineralization was achieved with a daily intake of 4-4.5g non-phytate phosphorus, which was lower than the requirement of 5.5g for growth.

The lack of response of femur calcium content was surprising in view of the changing calcium-to-phosphorus ratio in the experimental diets. The mean Ca:P ratio in the femur, at 2.44, was higher than the generally accepted value of just greater than 2:1 (Whittemore, 1970). Lack of response of third metatarsal mineral content to either phosphorus level or to phytase confirmed the suggestion (Crenshaw *et al*, 1981) that these bones were unsuitable as indicators of phosphorus availability in very young pigs. In contrast, total ash and total phosphorus of the forth metatarsal responded to phosphorus intake, which was unexpected since strength of the bone did not show a concomitant increase. It may be that a dramatic increase in mineralization would be required in order for strength to be affected. Alternatively, perhaps the low Ca:P ratio at high levels of dietary phosphorus reduced the strength of the mineralized bone. Percent of ash in the metatarsals was somewhat lower than that found by Young *et al.*, 1993 and Mahan (1982) who observed 53%-54% ash in pigs of 10-20kg liveweight fed a dietary phosphorus level of 0.68%. That this difference could be explained by the low phosphorus levels in some of the diets used seems questionable; in many treatments the use of phytase and increased levels of inorganic phosphorus should have been adequate for maximum ash accretion.

Although addition of phytase to the diets resulted in an increased total phosphorus in the femur at all levels of dietary phosphorus, percent of phosphorus in the femur was not affected. Similarly, total ash in the femur was strongly influenced by phytase addition, but percent of ash only weakly so. This suggested that changes in total phosphorus in the femur were due an increased size of the bones rather than solely to increased mineralization. In another trial using pigs of a similar size, phytase supplementation to a diet containing 0.5% phosphorus resulted in an increased weight, ash content, and total phosphorus of bones; and responses to phytase were no different to those obtained by increasing dietary phosphorus (Young *et al.*, 1993). It was suggested that the observed changes in bone traits may have been a result of increased growth rate of pigs fed inorganic phosphate or phytase.

Increase in size or mineralization enables a bone to withstand a greater force. Thus whether the pigs receiving phytase achieved an increased bone size or increased mineralization; the resultant increase in bone strength was the important outcome. It may have been that pigs receiving the enzyme were subjected to stimulated growth over and above that caused by increasing phosphorus intake alone, thus phosphorus was needed for the production of new bone mass rather than for more intensive mineralization of existing bone mass. On the other hand, one would expect that in pigs of this age, hormonal mechanisms would already be directed towards new bone growth, rather than intensive mineralization of existing bone.

At the ends (epiphyses) of the bones there is persistent and rapid growth until the bone reaches adult size. At the same time, bone resorption occurs from the inner surfaces. The long bones characteristically grow in length much faster than they do in width (Simkiss, 1975). Present results indicated no response of bone length either to phosphorus or phytase, however it is possible that some differences may have been detected had pigs been slaughtered at equal ages, rather than at equal weights.

From the regression analyses it was calculated that approximately one third of digested phosphorus contributed towards mineralization (table 6.65). Based on analysis of variance data it was calculated that addition of phytase to the basal diet made approximately 70% of the phytate-phosphorus available for bone accretion (Table 6.66). As seen with growth and bone strength, addition of phytase seemed to "mask" the regression responses of femur mineral against phosphorus intake.

Table 6.65 Calculation of dietary phosphorus contributing to mineralization

0.21g P in femur required for 10 Newton increase in strength
0.6g P in diet required for 10 Newton increase in strength

Therefore 0.6 g P in diet => 0.21g P in femur
Amount of P in diet required for 1g P in femur = 0.6 * 1/0.21
= 3.5g

Table 6.66 Calculation of phytate-phosphorus made available by phytase

Non-phytate P (N-P-P) in basal diet = 2.0 g/kg
Total P in basal diet = 4.2 g/kg
Phytate P = 2.2 g/kg
DLWG at 2.0 g/kg N-P-P (+ phytase) ≡ DLWG at 3.5g/kg N-P-P (-phytase)
Whole body DMP% " " ≡ carcase P at 3.5g/kg N-P-P (-phytase)
Femur strength " " ≡ strength at 3.5g/kg N-P-P (-phytase)
Femur P content " " ≡ P content at 3-3.5g/kg N-P-P (-phytase)

Assuming that all extra phosphorus has become available through the use of phytase,
Phosphorus made available through phytase: = 3.5 - 2.0
= 1.5g/kg
As proportion of phytate phosphorus = (1.5/2.0)*100
= 75%

Table 6.67 Daily digestible phosphorus required to achieve maximum response

Criteria of response	*Daily N-P-P intake required for maximum response (g)*
Daily gain	5.5
Daily feed intake	6.5
Total phosphorus in body	5.5
Percent phosphorus in body	5.5
Femur strength	4
Total phosphorus in femur	4
Ash content of femur	4.5

A total dietary concentration of 0.68%P to maximise bone ash, 0.58% to maximise performance traits, and an available phosphorus content of 0.35% was suggested by Mahan (1982). Based on present results of a daily requirement of 5.5g of digestible phosphorus, and a daily feed intake of 1.48kg freshweight, a dietary concentration of 3.7 g digestible phosphorus would be required for pigs of 10-25kg.

Recommendations of phosphorus requirements by the National Research Council and the Agricultural Research Council are a compromise to achieve maximum growth and adequate bone development. However, having decided that growth responses are not sensitive to phosphorus intake, it seems probable that the requirement for maximum growth determined by the ARC is somewhat inaccurate.

In comparing responses against intake of non-phytate phosphorus to those against intake of total phosphorus, similar sensitivities were observed; indeed in some cases, response to daily total phosphorus was slightly more sensitive than that to daily non-phytate phosphorus intake (eg for the quadratic response of average daily, percentage variation accounted for was 21.7 when regressed against non-phytate phosphorus, vs 24.2 when regressed against total phosphorus intake). This was surprising since it was presumed that non-phytate phosphorus, in giving a more reliable indication of available dietary phosphorus, would show superior responses than total phosphorus. Sensitivity of the responses may have been due in part to those diets containing a high ratio of inorganic:organic phosphorus, where the regressions against total phosphorus would have tended towards those against non-phytate-phosphorus.

6.4.6 Gender differences

Boars had a higher percentage of phosphorus and ash in their bodies. Their femurs were heavier and strength was more responsive to phosphorus than those of gilts. Conversely, femurs of gilts contained a greater concentration of ash. In an earlier experiment it was observed that bones from gilts withstood more stress than those from barrows and boars (Crenshaw 1986), although in the present experiment, no effects of gender on femur strength were apparent.

The combined evidence suggested that boars directed their phosphorus intake more towards growth; mineralization occurring in order to increase size, rather than density, of the bones, whereas gilts, in striving towards mineralization of the bones, tended to accumulate phosphorus in the bones, perhaps at the expense of using phosphorus for growth. On a biological basis this explanation would be justified in that gilts require a skeletal reserve of phosphorus for use during future pregnancy and lactation, and it would seem sensible to suggest that their hormonal mechanisms were tuned to target phosphorus towards the skeleton. This suggestion is made in spite of the lack of difference in growth rate between sexes; it may have been that differences in growth rate between the sexes did not come into effect at such small sizes.

Boars had a higher concentration of phosphorus and ash in their body than gilts, in contrast to previous work by Mudd *et al.* (1969) which showed no difference between sexes. It has been reported that maximum bone strength of boars requires levels above the current NRC recommended levels for optimum growth (Nimmo *et al.*, 1980). Based on present observations it seems likely that boars which are growing at their optimum rate will be using dietary phosphorus to maintain growth rate, rather than bone mineralization. In contrast to the present results, work of Maxson and Mahan (1983) showed no differences in bone strength or bone ash of growing pigs.

The number of animals used (2 boars and 2 gilts) for each of the dietary treatments was a compromise between looking at responses with and without the enzyme, and looking at the requirement for phosphorus. The former was achieved through analysis of variance, which required sufficient animals per treatment to detect statistical significance, whereas the response analysis by regression, depended on a large number of treatments (ie dietary phosphorus levels), with few animals per treatment. Differences due to gender that were apparent meant that the statistical power of the experiment was lower than it would have been had the boars and gilts responded similarly, but on the other hand, also meant that the results were representative of a true population.

6.4.8 Phosphorus/phytase equivalence

Although in designing the experiment, it was assumed that regression responses both with and without the enzyme could be established, addition of phytase destroyed the linear and curvilinear responses to phosphorus intake. This suggested some disruption of the homeostatic mechanism of pigs receiving phytase, or an interaction of inorganic phosphorus with phytase with the net result of different amounts of phosphorus being made digestible, which would subsequently be reflected in growth and bone development. Despite this, by comparing response criteria at the basal level of phosphorus with and without the enzyme, it could be seen that approximately 70% of the phytate-phosphorus was being made available for digestion, and this estimate was consistent whether based on growth, bone strength or mineral composition.

The amount of phytate-phosphorus made available by the use of phytase was greater than the 33% phytate-availability observed by Cromwell *et al.* (1993), and than results of Young *et al.* (1993) who observed that phytase addition to diets containing approximately 0.56% phosphorus was as effective as 0.75% phosphorus obtained with calcium phosphate. Present estimates were also higher than the 50% increase in ileal digestibility observed in the previous digestibility trial using cannulated pigs (*Section 3.3*). Due to the very low basal level of phosphorus used in the present experiment it is probable that the effect of phytase was more pronounced. This should be taken into consideration when contemplating the use of phytase; the enzyme will be used more efficiently in diets which do not already contain a level of inorganic phosphorus.

6.5 Conclusions

A daily intake of 5.5g digestible phosphorus was necessary for maximum growth. However, 4g digestible phosphorus/day was sufficient for maximum bone strength of young pigs; any further intake interfering with mineralization to decrease bone strength. Adding phytase to a phytate-rich diet at low levels of digestible phosphorus made approximately 70% of the phytate-phosphorus available for bone accretion.

Chapter 7 Experiment 4 - Effect of phosphorus level and phytase on growth and bone development of growing pigs

7.1 Introduction

Genetic improvement in recent years has produced a fast growing, lean, pig. However, appetite of the modern genotype has not increased to support the improved growth, and consequently diets must now be more concentrated, to match requirements for fast growth. Inconsistencies in estimated phosphorus requirements of growing pigs between the various research councils have been the cause of much debate, particularly in light of the environmental constraints imposed on the livestock production sector. Growing pigs contribute enormously to total phosphorus output from the farming sector; annual phosphate output from pigs is estimated at 47,000 tonnes (Lee and Tucker, 1994). The livestock and associated feed industries, under increasing public and political pressure, have been compelled to give more consideration to the environment, and must now actively reduce phosphate output.

The 5-10% 'safety margin' of phosphorus allowance is no longer appropriate, instead the dietary phosphorus level must match more accurately the requirement. Values of a net requirement estimated by the ARC (1981) extend from 4.6 g/day for 25kg liveweight, to 5.2g/day from 45kg liveweight. In extreme contrast, the NRC (1988) recommend 2.3 g/kg available phosphorus in the diet of growing pigs. Values lying between these have also been recommended. Changes in requirements within the growth period and changes in requirements due to differences in growth rate should also be more fully investigated. Despite claims of reduced nitrogen excretion as a result of phase-feeding, which could apply also to phosphorus excretion, many pig producers still feed the same level of phosphorus throughout the growth period.

In view of the inconsistencies in recommended dietary phosphorus intakes, and particularly because of the environmental pressure, a re-evaluation of dietary phosphorus requirements seems appropriate.

Phosphorus already present in the diet should also be made available to the pig. The use of phytase to improve the availability of phytate-phosphorus in the diets of growing pigs is well documented. But the question "how much inorganic phosphorus can be replaced by phytase?" remains unsolved, having so far been addressed only as a secondary observation (eg Cromwell *et al.*, 1993).

To date, effects of phytase on carcase traits are unknown. But if it is assumed that phytase influences growth rate, by effectively altering the availability of phosphorus, it seems reasonable to suggest that this may be reflected in an alteration of lean tissue growth. Animals growing faster due to phytase or a high level of dietary phosphorus would be expected to be leaner at a given slaughter weight than slower growing animals receiving low levels of dietary phosphorus.

In pigs reared for slaughter at 100kg or less, maximum bone strength is not necessary. It can, however be used as an indicator of phosphorus availability, and if measured concomitantly with growth performance would give an indication of phosphorus requirement. The objectives of this work were therefore to look at the phosphorus requirement of growing pigs (25-60kg), and to assess the effect of phytase on phosphorus availability using growth and bone development as criteria of response.

7.2 Materials and Method

7.2.1 Experimental Design

A total of 72 animals was used, of single sex (boars) in order to avoid variation due to gender. 9 dietary levels of non-phytate phosphorus were fed either with or without phytase, totalling 18 diets. Each was fed *ad libitum* to 4 pigs.

7.2.2 Diet

Formulation and nutrient specification of the diet are shown in tables 7.1 and 7.2 respectively. The base level of phosphorus was 2.5g/kg, 0.85g/kg of which was non-phytate phosphorus. By incremental addition of mono-ammonium phosphate, levels of non-phytate phosphorus were increased by 0.4 g/kg as in table 7.3. The 18 treatments consisted of 9 levels of phosphorus with phytase, and the same 9 levels without phytase. Activity of the enzyme was 300 units/g. The enzyme was premixed into the appropriate diets at an inclusion rate of 3.33 g/kg. This provided approximately 1000 units of activity per kg freshweight of feed.

7.2.3 Procedure

The procedure as described in *section 3.5.3* of the General Materials and Methods was followed. During the experiment feed and water were available *ad libitum*. Each pig started the trial upon reaching 25kg liveweight and ended the trial at 60kg liveweight. At slaughter, the third and fourth metatarsals were removed from the left hind leg and stored at -18°C. Left side carcase measurements were taken 24 hours after slaughter.

Physical measurements of the left third and fourth metatarsals (freshweight, longitudinal length and breaking strength) were determined on the defrosted "fresh" bones. Thereafter, ash and phosphorus content of the fat free bone were determined.

7.2.4 Statistical analysis

Growth data were subjected to regression analysis to obtain average daily gains of the pigs. Mean daily feed intake, average daily liveweight gain and feed conversion data were analyzed using analysis of variance (Genstat 5, Lawes Agricultural Trust) to test for the effects of phosphorus level and phytase. Analysis of covariance using 'slaughterweight' as the covariate was performed for carcase measurements, bone physical measurements and bone mineral data.

Regression analysis was performed for all parameters against daily non-phytate phosphorus intake and daily total phosphorus intake. Linear and curvilinear lines were fitted through the data to obtain the nature of the responses. Finally, relationships between the various response parameters (eg bone mineral content vs bone strength, growth rate vs carcase measurements) were tested using linear regression analysis.

Table 7.1 Formulation and phosphorus/phytate content of experimental diet

Ingredient	g/kg	phosphorus (g/kg FW)	total phosphorus contribution to diet	phytate-phosphorus (%)	phytate-phosphorus contribution to diet
Maize	400	2.6	1.04	66	0.70
Oatfeed	282.5	-	-	-	-
Soya 50	220	6.8	1.50	61	0.92
Vegetable oil	69	-	-	-	-
Limestone	11	-	-	-	-
Betamix 314	12.5	-	-	-	-
Synthetic lysine	3.5	-	-	-	-
Threonine	0.7	-	-	-	-
Synthetic methionine	0.6	-	-	-	-
Synthetic tryptophan	0.2	-	-	-	-
Total	1000		2.5		1.65

Table 7.2 Nutrient specification of basal diet

Nutrient	g/kg Freshweight
Dry matter	888
Digestible energy (MJ/kg)	13.4
Crude protein	163.89
Oil	86.20
Crude fibre	94.17
Lysine	10.80
Methionine	2.86
Methionine + cystine	5.40
Threonine	6.50
Tryptophan	1.95
Phenylalanine + tyrosine	13.18
Ash	29.88
Calcium	8.00
Phosphorus	2.50
Salt	2.76
Linoleic acid	43.4

Table 7.3 Phosphorus and phytase content of experimental diets

Diet	g/kg non-phytate P	g/kg total P	+/- phytase
1 (basal)	0.85	2.5	-
2	1.25	2.9	-
3	1.65	3.3	-
4	2.05	3.7	-
5	2.45	4.1	-
6	2.85	4.5	-
7	3.25	4.9	-
8	3.65	5.4	-
9	4.05	5.9	-
10	0.85	2.5	+
11	1.25	2.9	+
12	1.65	3.3	+
13	2.05	3.7	+
14	2.45	4.1	+
15	2.85	4.5	+
16	3.25	4.9	+
17	3.65	5.4	+
18	4.05	5.9	+

7.3 Results

7.3.1 Health of the pigs

Throughout the trial there were some infectious scouring problems leading to a reduced feed intake. These were treated with the antibiotic depocillin. A full record is given in appendix IV. In all, 6 pigs were treated of which 5 recovered and 1 was removed from the experiment. There were no obvious locomotory problems except for one pig which developed a severe lameness with additional scouring problems. This pig did not respond to treatment with lincocin antibiotic and was removed from the trial. Two further pigs were taken off the trial, one of which developed a scouring problem towards the end of the trial, and the other because it refused feed with no indication of an infection. In total four pigs were removed from the experiment, leaving 68 out of a total of 72 pigs to finish the trial.

7.3.2 Growth performance

Effects of phosphorus level, phytase, and the interactions on feed intake, daily liveweight gain and feed conversion efficiency are summarised in table 7.4. Comparison of sed values with the treatment means showed an effect of phytase on growth at the lowest of dietary phosphorus, despite the non-significant probability value obtained. Further analysis of enzyme effects between diets 1 (basal diet) and 10 (basal diet + phytase) confirmed an effect of the enzyme at the lowest level of dietary phosphorus (table 7.5). A lowered daily liveweight gain of pigs receiving the basal diet (0.85 g/kg non-phytate phosphorus) was overcome by addition of phytase, and approached that of pigs receiving the 1.65 g/kg non-phytate phosphorus diet (figure 7.1). Responses of liveweight gain to phosphorus level and phytase appeared to be mainly due to differences in feed intake (figure 7.2), although feed intake differences were statistically non-significant. Pigs fed the basal diet with supplementary phytase used feed less efficiently than those without, but at other levels there were no differences except at 3.25-3.65 g/kg non-phytate-phosphorus, where phytase supplemented diets were again less efficient (figure 7.3). At very low (0.85 g/kg) and very high (4.05 g/kg) levels of non-phytate phosphorus, poor conversion ratios were seen both with and without the enzyme.

Regression analysis revealed linear and quadratic responses of daily liveweight gain to daily intake of digestible phosphorus (figures 7.4 and 7.5 respectively), and a strong quadratic response of feed conversion efficiency to daily intake of digestible phosphorus. Responses to daily intake of total phosphorus were also apparent. The significant relationships are presented mathematically in table 7.6. Growth performances of individual animals are presented in appendix V.

Figure 7.1 Effect of phosphorus level and phytase on daily liveweight gain of growing pigs

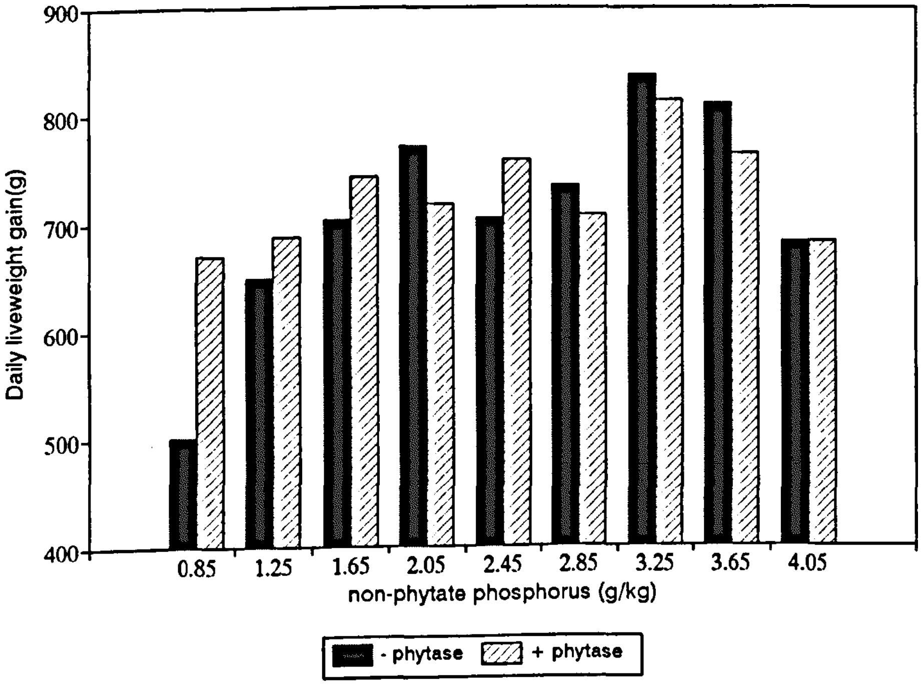


Figure 7.2 **Effect of phosphorus level and phytase on daily feed intake of growing pigs**

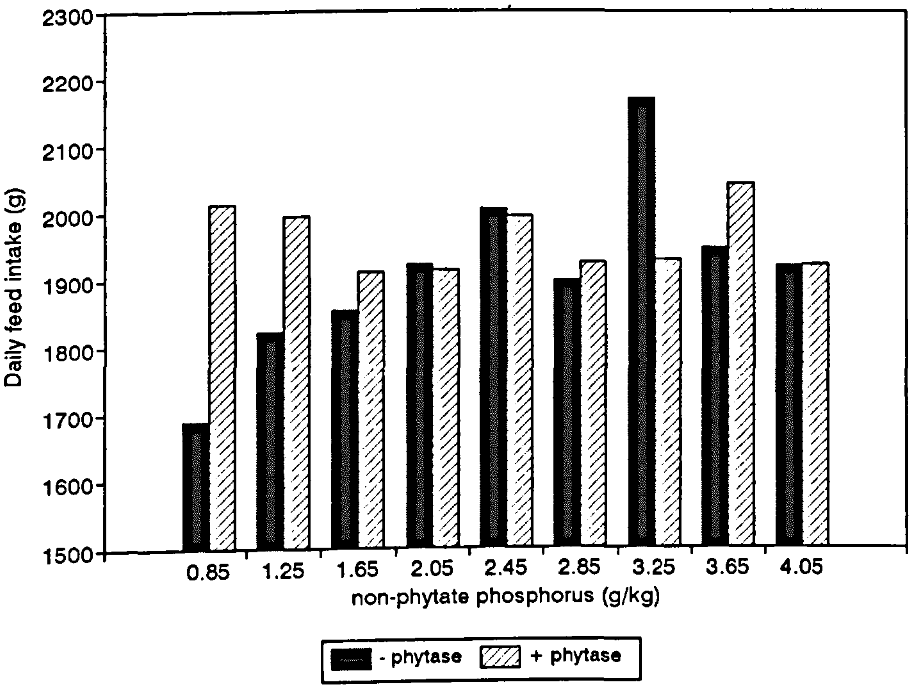


Figure 7.3 Effect of phosphorus level and phytase on feed conversion ratio of growing pigs

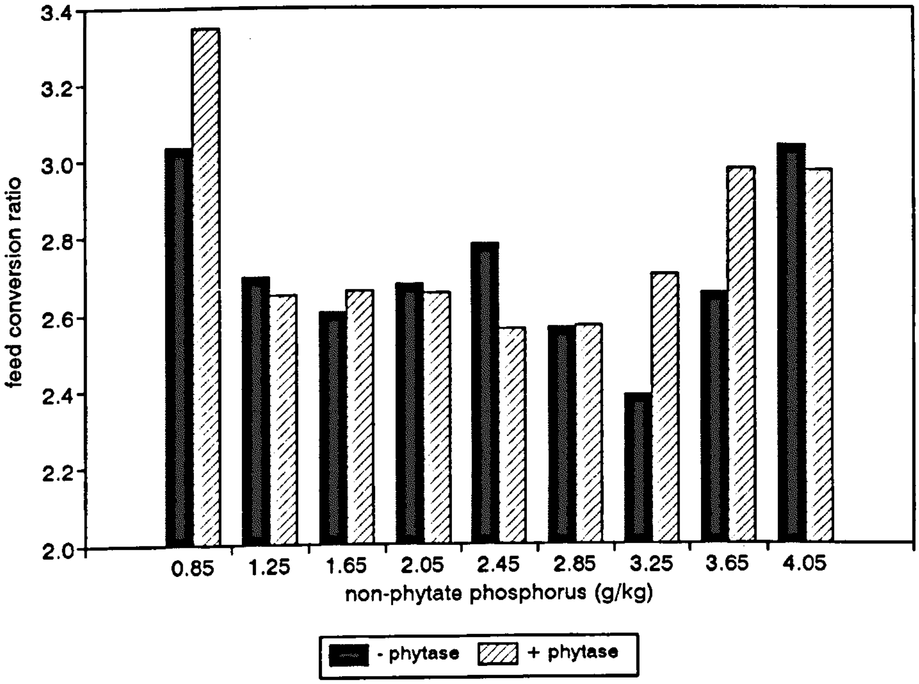


Table 7.4 Significance of effects of phosphorus level, phytase and the interactions on growth performance

	daily feed intake		*daily liveweight gain*		*feed conversion ratio*	
	p	sed	p	sed	p	sed
Phosphorus level	ns	0.134	0.005	0.055	0.004	0.168
Phytase	ns	0.063	ns	0.026	ns	0.079
Phosphorus level * phytase	ns	0.189	ns	0.078	ns	0.238

Table 7.5 Effect of adding phytase to the basal diet on daily liveweight gain

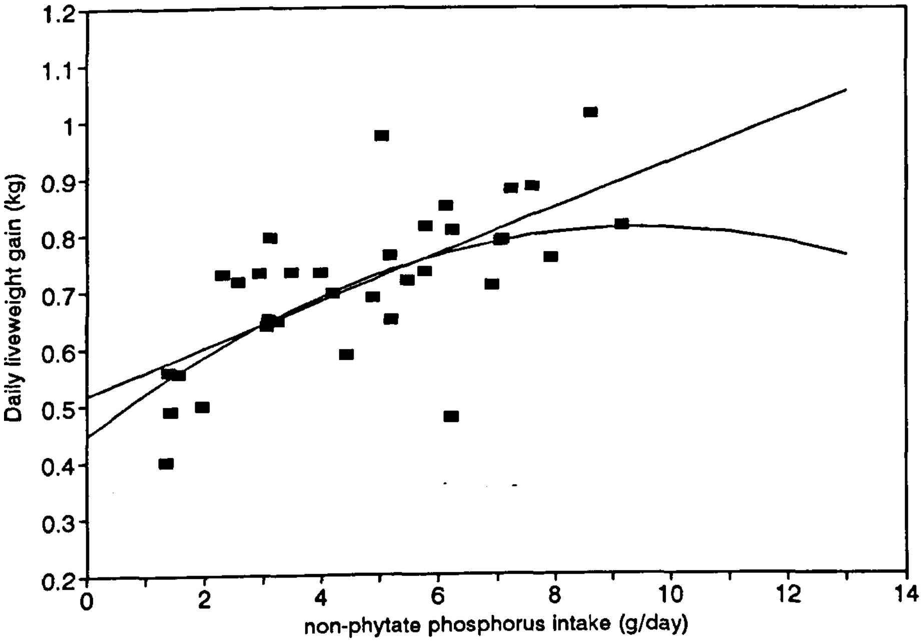
daily liveweight gain		p	sed
- phytase	+ phytase		
0.501	0.669	0.015	0.050

Table 7.6 Regression of growth parameters (y) against phosphorus intake (x)

	Linear			*Quadratic*		
	Equation of response	p	%	Equation of response	p	% var[τ]
Daily non-phytate phosphorus intake (g)						
Daily feed intake (kg)	-	ns	-	-	ns	-
Daily liveweight gain (kg)	$y = 0.518 + 0.041x$	<0.001	52.0	$y = 0.449 + 0.076x - 0.004x^2$	<0.001	42.7
Feed conversion ratio	$y = 3.037 - 0.061x$	0.029	11.3	$y = 3.54 - 0.32x + 0.027x^2$	0.009	21.7
Daily total phosphorus intake (g)						
Daily feed intake (kg)	-	ns	-	-	ns	-
Daily liveweight gain (kg)	$y = 0.397 + 0.040x$	<0.001	52.0	$y = 0.226 + 0.75x + 0.002x^2$	<0.001	51.5
Feed conversion ratio	$y = 3.175 - 0.054x$	0.029	11.3	$y = 4.35 - 0.37x + 0.020x^2$	0.011	20.6

[τ] % variance accounted for

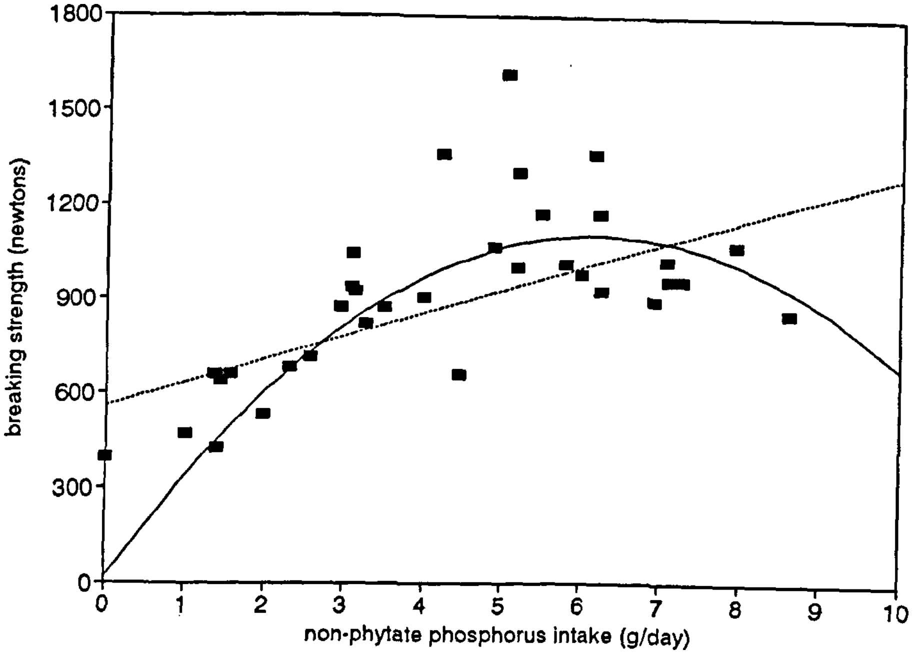
Figure 7.4 **Response of daily liveweight gain (y) to daily phosphorus intake (x)**



Response equations:

Linear	$y = 0.518 + 0.041x$	$p < 0.001$	%var 52.0
Quadratic	$y = 0.449 + 0.076x - 0.004x^2$	$p < 0.001$	%var 42.7

Figure 7.5 Response of feed conversion ratio (y) to daily phosphorus intake (x)



Response equations:

Linear $y = 3.037 + 0.061x$ $p = 0.029$ %var 11.3

Quadratic $y = 3.54 - 0.32x + 0.027x^2$ $p = 0.009$ %var 21.7

7.3.3 Carcase measurements

Significant effects of phosphorus level, phytase and the interactions on slaughter measurements are shown in table 7.7, and treatment means are presented in tables 7.8- 7.21. Regression analysis performed on the data showed a linear relationship between daily intake of non-phytate/ total phosphorus and the cross-sectional width (A) of the *Longissimus dorsi* muscle. Carcase weight and fat depth (maximum shoulder, minimum midback, anterior *gluteus medius*, posterior *gluteus medius*, and minimum *gluteus medius*) were unaffected by the treatments.

While there were few relationships between daily phosphorus intake and measurements of carcase fat, when phytase was added relationships some weak relationships became apparent (ie P1, P2 and C). These are described in table 7.22 and 7.23

Table 7.7 Significance of effects of phosphorus level and phytase on carcase measurements

variate	factor	p	sed
Carcase Length	P level * phytase	0.088	12.67
Width of *L. Dorsi* (A)	P level	0.055	2.32
Breadth of *L. Dorsi* (B)	P level	0.036	1.72

Table 7.8 Mean slaughter weight (kg) of the treatment groups

Phosphorus (g/kg)	- phytase	+ phytase
0.85	60.75	63.25
1.25	61.17	64.50
1.65	63.12	63.75
2.05	62.00	63.37
2.45	61.37	62.75
2.85	62.87	62.12
3.25	62.12	65.12
3.65	62.50	62.00
4.05	64.00	62.00

Table 7.9 Mean carcase weight (kg) of the treatment groups

Phosphorus level[r]	- phytase	+ phytase
0.85	44.60	43.92
1.25	42.86	43.31
1.65	44.45	44.35
2.05	43.75	44.93
2.45	44.32	43.75
2.85	44.48	44.58
3.25	43.93	44.57
3.65	42.83	43.85
4.05	43.36	44.33

Table 7.10 Mean carcase length (mm) of the treatment groups

Phosphorus (g/kg)	- phytase	+ phytase
0.85	696.4	695.1
1.25	684.5	715.5
1.65	693.2	686.5
2.05	715.5	686.5
2.45	685.9	702.2
2.85	696.7	689.9
3.25	686.6	694.3
3.65	683.0	698.6
4.05	692.3	697.7

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[r] non-phytate phosphorus g/kg feed

Table 7.11 Mean maximum shoulder (mm) of the treatment groups

Phosphorus (g/kg)	- phytase	+ phytase
0.85	26.66	26.88
1.25	28.14	27.33
1.65	25.51	26.96
2.05	27.26	25.32
2.45	25.51	27.31
2.85	23.64	24.63
3.25	24.45	24.21
3.65	25.12	26.11
4.05	24.26	26.13

Table 7.12 Mean minimum midback (mm) of the treatment groups

Phosphorus (g/kg)	- phytase	+ phytase
0.85	8.33	8.26
1.25	8.53	5.42
1.65	8.65	7.07
2.05	9.05	10.06
2.45	8.29	8.75
2.85	10.57	8.11
3.25	7.76	8.17
3.65	9.13	8.48
4.05	8.18	8.99

non-phytate phosphorus g/kg feed

Table 7.13 Mean anterior *gluteus medius* (mm) of the treatment groups

Phosphorus (g/kg)	- phytase	+ phytase
0.85	12.50	10.95
1.25	10.00	10.59
1.65	11.53	12.39
2.05	12.49	10.93
2.45	12.79	10.52
2.85	12.02	12.11
3.25	9.34	8.45
3.65	9.75	11.73
4.05	9.94	9.39

Table 7.14 Mean minimum *gluteus medius* (mm) of the treatment groups

Phosphorus (g/kg)	- phytase	+ phytase
0.85	5.34	3.71
1.25	6.78	5.81
1.65	6.79	7.62
2.05	7.50	6.15
2.45	6.79	4.87
2.85	6.05	6.84
3.25	4.98	4.14
3.65	6.83	8.79
4.05	4.50	6.44

[†] non-phytate phosphorus g/kg feed

Table 7.15 Mean posterior *gluteus medius* (mm) of the treatment groups

Phosphorus (g/kg)	- phytase	+ phytase
0.85	11.04	9.47
1.25	11.46	12.68
1.65	9.30	11.54
2.05	10.96	11.55
2.45	10.88	10.41
2.85	9.64	11.90
3.25	11.58	9.79
3.65	10.52	13.65
4.05	7.56	10.65

Table 7.16 Mean P1 (mm) of the treatment groups

Phosphorus (g/kg)	- phytase	+ phytase
0.85	5.93	5.39
1.25	7.17	6.80
1.65	7.37	6.75
2.05	6.82	5.98
2.45	6.72	7.27
2.85	5.90	5.79
3.25	5.20	6.14
3.65	6.71	7.61
4.05	7.11	7.02

' non-phytate phosphorus g/kg feed

Table 7.17 Mean P2 (mm) of the treatment groups

Phosphorus (g/kg)	- phytase	+ phytase
0.85	6.02	5.05
1.25	6.84	6.06
1.65	6.43	6.47
2.05	6.72	5.59
2.45	6.20	6.40
2.85	5.95	5.15
3.25	5.28	5.59
3.65	5.66	7.32
4.05	6.15	6.20

Table 7.18 Mean P3 (mm) of the treatment groups

Phosphorus (g/kg)	- phytase	+ phytase
0.85	5.90	5.25
1.25	7.45	6.49
1.65	6.36	7.30
2.05	6.97	6.82
2.45	7.03	7.47
2.85	6.40	5.83
3.25	5.08	5.78
3.65	6.16	7.86
4.05	6.38	7.13

[τ] non-phytate phosphorus g/kg feed

Table 7.19 Mean "A" measurement (mm) of the treatment groups

Phosphorus (g/kg)	- phytase	+ phytase
0.85	87.32	85.53
1.25	79.85	77.72
1.65	84.95	80.61
2.05	83.86	85.78
2.45	84.50	81.56
2.85	82.74	83.55
3.25	81.61	83.36
3.65	80.77	78.70
4.05	83.55	84.70

Table 7.20 Mean "B" measurement (mm) of the treatment groups

Phosphorus (g/kg)	- phytase	+ phytase
0.85	48.88	47.93
1.25	46.46	42.21
1.65	46.29	45.86
2.05	48.11	48.39
2.45	46.81	46.14
2.85	46.66	51.84
3.25	47.20	52.26
3.65	45.68	44.66
4.05	47.40	46.39

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[r] non-phytate phosphorus g/kg feed

Table 7.21 Mean "C" measurement (mm) of the treatment groups

Phosphorus (g/kg)	- phytase	+ phytase
0.85	6.05	5.06
1.25	6.97	6.09
1.65	6.42	6.45
2.05	6.45	5.41
2.45	6.01	7.02
2.85	5.72	5.33
3.25	5.33	5.60
3.65	5.75	7.15
4.05	5.99	6.56

† non-phytate phosphorus g/kg feed

Table 7.22 Linear regression of carcase measurements (y) against phosphorus intake (x) without (-) or with (+) phytase

	- phytase			*+ phytase*		
	Equation of response	p	% var[τ]	Equation of response	p	% var[τ]
Daily non-phytate phosphorus intake						
A	y = 86.5 - 0.654x	0.057	8.1	-	ns	-
MM	-	ns	-	y = 6.71 + 0.322x	0.092	5.8
P1	-	ns	-	y = 5.26 + 0.261x	0.052	8.5
P2	-	ns	-	y = 4.86 + 0.232	0.068	7.2
C	-	ns	-	y = 4.90 + 0.246x	0.042	9.5
Daily total phosphorus intake						
A	y = 88.42 - 0.636	0.036	10.30	-	ns	-
MM	-	ns	-	y = 5.41 + 0.354	0.045	9.2
P1	-	ns	-	y = 4.30 + 0.276	0.026	11.9
P2	-	ns	-	y = 3.88 + 0.261	0.027	11.8
P3	-	ns	-	y = 4.59 + 0.258	0.063	7.6
C	-	ns	-	y = 3.94 + 0.267	0.017	13.8

[τ] percentage variance accounted for

Table 7.23 **Non-linear regression of carcase measurements (y) against phosphorus intake (x) without (-) or with (+) phytase**

	- phytase			*+ phytase*		
	Equation of response	p	% var$^{\tau}$	Equation of response	p	% var$^{\tau}$
Daily non-phytate phosphorus intake						
P1	-	ns	-	$y = 7.89 - 0.10x + 0.124x^2$	0.015	18.8
P2	-	ns	-	$y = 7.10 - 0.84x + 0.106x^2$	0.031	15.0
C	-	ns	-	$y = 7.06 - 0.79x + 0.102x^2$	0.052	17.5
Daily total phosphate intake						
P1	-	ns	-	$y = 12.15 - 1.713x + 0.117x^2$	0.004	25.6
P2	-	ns	-	$y = 10.02 - 1.295x + 0.091x^2$	0.011	20.2
P3	-	ns	-	$y = 8.86 - 0.823x + 0.063x^2$	0.094	8.6
A	$y = 81.23 + 1.31x - 0.12x^2$	0.068	10.5	-	ns	ns
C	-	ns	-	$y = 9.98 - 1.263x + 0.090x^2$	0.007	23.0

τ percentage variance accounted for

7.3.3 Physical properties of the metatarsal bones

Effects of phosphorus level, phytase and the interactions on the physical properties of the third and fourth metatarsals are presented in table 7.24. Both phosphorus level and enzyme influenced the freshweight and the breaking strength of the third and fourth metatarsals. In both the third and fourth metatarsals, increases in bone breaking strength with the enzyme were achieved at levels of up to 2.45 g/kg non-phytate phosphorus (figure 7.6 and 7.7). Length of the bones was unaffected either by dietary phosphorus level or phytase. Treatment group means are shown in tables 7.25-7.27.

Strong linear and quadratic responses of metatarsal strength to increasing daily intake of non-phytate/total phosphorus were observed; as presented in figures 7.8 and 7.9. Response equations are shown in table 7.28. The fourth metatarsal appeared to be more responsive to phosphorus level than the third, exhibiting a greater increase in strength per unit increase in dietary phosphorus. Addition of phytase destroyed all linear and quadratic relationships between strength and phosphorus intake.

Figure 7.6 Effect of phosphorus level and phytase on breaking strength of the third metatarsal

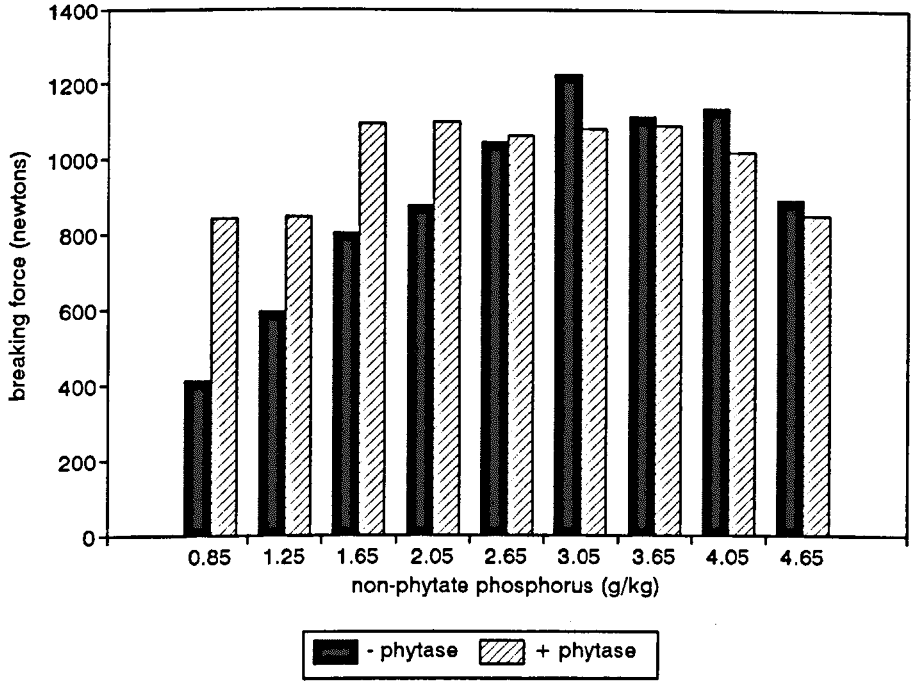


Figure 7.7 Effect of phosphorus level and phytase on breaking strength of the fourth metatarsal

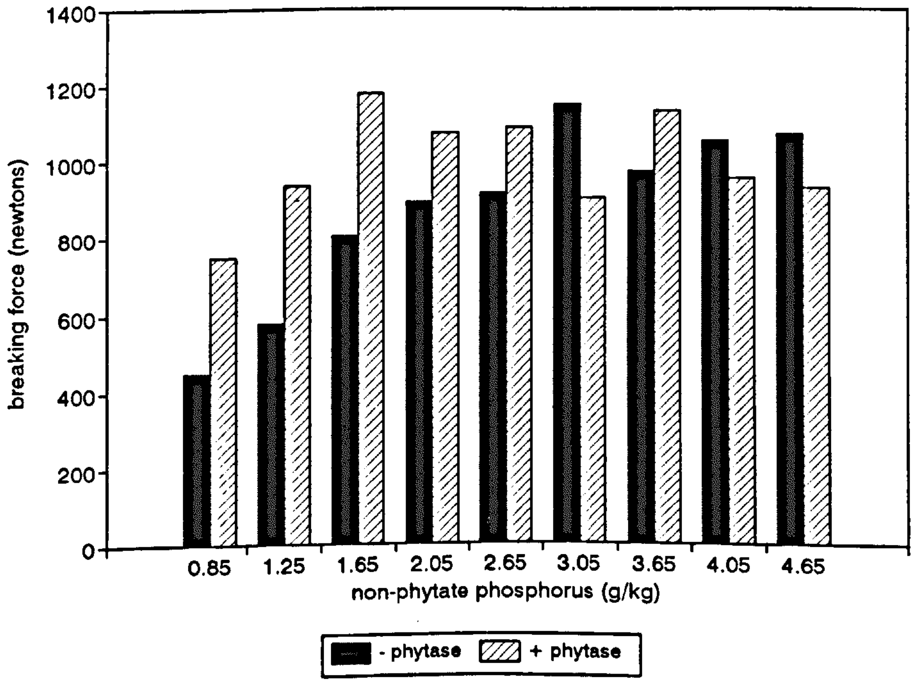


Table 7.24 Significance of effects of phosphorus level, phytase and the interactions on physical properties of the metatarsals

Variate	Factor	p	sed
3rd metatarsal			
Freshweight	enzyme	0.054	0.721
	P level x enzyme	0.034	2.169
Length	ns	>0.10	-
Strength	P level	<0.001	100.7
	enzyme	0.055	47.4
4th metatarsal			
Freshweight	enzyme	0.028	0.68
	P level x enzyme	0.044	2.03
Length	ns	>0.10	-
Strength	P level	<0.001	91.2
	enzyme	0.006	42.9
	P level x enzyme	0.007	129.3

Table 7.25 Mean metatarsal freshweight (g) of the treatment groups

P level[r]	3rd metatarsal		4th metatarsal	
	- phytase	+ phytase	- phytase	+ phytase
0.85	23.61	28.57	24.04	28.29
1.25	24.58	24.29	27.00	23.58
1.65	26.29	25.12	25.59	24.26
2.05	24.71	30.28	25.11	28.63
2.45	22.82	28.28	22.61	28.18
2.85	25.00	22.22	24.12	24.53
3.25	24.11	27.20	25.88	27.09
3.65	26.58	26.62	24.77	28.77
4.05	27.12	25.00	26.94	26.47

Table 7.26 Mean metatarsal length (mm) of the treatment groups

P level[r]	3rd metatarsal		4th metatarsal	
	- phytase	+ phytase	- phytase	+ phytase
0.85	71.17	72.29	73.32	75.21
1.25	70.91	73.29	74.88	72.75
1.65	71.69	73.29	74.63	71.75
2.05	69.75	72.74	72.40	72.12
2.45	71.23	74.00	72.66	73.68
2.85	72.35	70.51	72.15	73.53
3.25	72.00	74.60	75.10	74.16
3.65	75.05	71.48	72.44	75.71
4.05	72.68	69.33	72.55	72.36

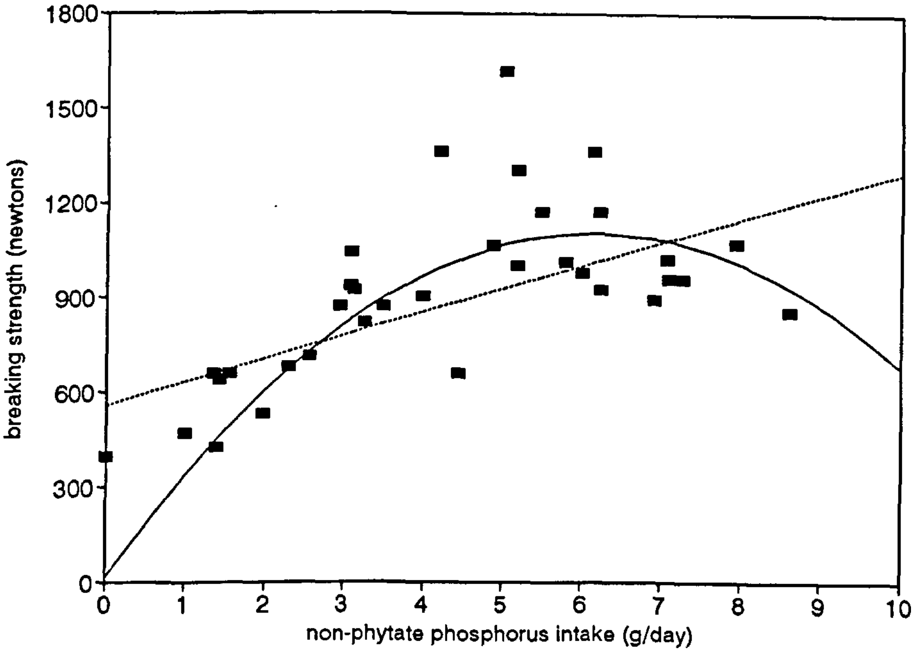
[r] non-phytate phosphorus g/kg feed

Table 7.27 Mean metatarsal breaking strength (newtons) of the treatment groups

P level[r]	*3rd metatarsal*		*4th metatarsal*	
	- phytase	+ phytase	- phytase	+ phytase
0.85	491	855	427	744
1.25	612	850	569	944
1.65	799	1090	804	1185
2.05	860	1099	889	1073
2.45	1039	1043	909	1092
2.85	1213	1071	1154	901
3.25	1067	1067	975	1140
3.65	1109	1003	1053	957
4.05	927	878	1063	921

[r] non-phytate phosphorus g/kg feed

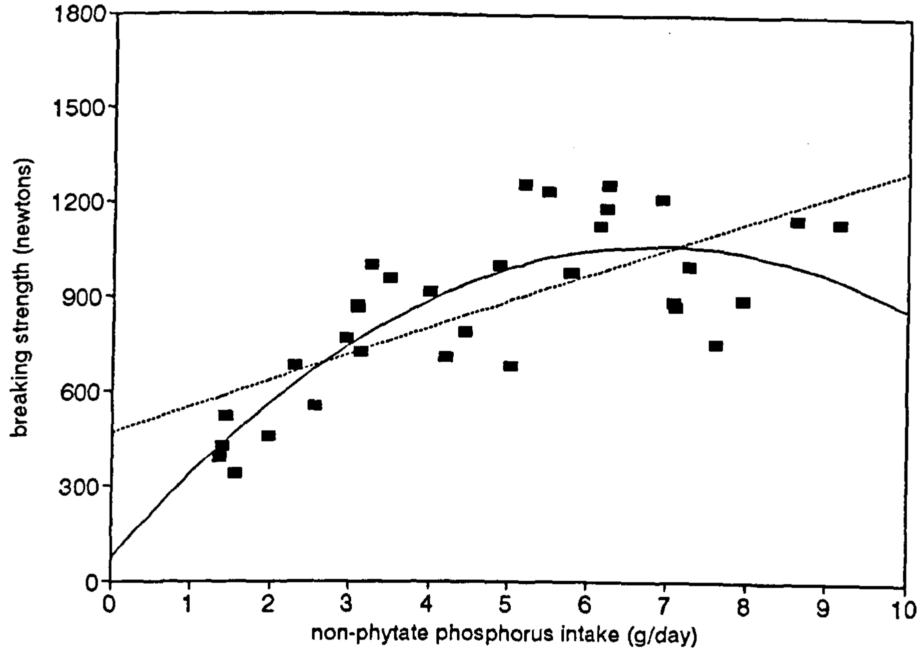
Figure 7.8 Response of third metatarsal breaking strength (y) to daily phosphorus intake (x)



Response equations

Linear	$y = 559.6 + 73.5x$	$p<0.001$	% var = 33
Quadratic	$y = 13 + 353.8x - 28.7x^2$	$p<0.001$	% var = 57.4

Figure 7.9 Response of fourth metatarsal breaking strength (y) to daily phosphorus intake (x)



Response equations

Linear	$y = 468.8 + 83.6x$	$p<0.001$	% var = 50.2
Quadratic	$y = y = 75 + 287.0x - 20.8x^2$	$p<0.001$	% var = 64

Table 7.28 Regression of metatarsal strength (y) against phosphorus intake (x)

	Linear			*Quadratic*		
	equation of response	p	% var	equation of response	p	% var
daily non-phytate phosphorus intake						
MT3 strength	$y = 559.6 + 73.5x$	<0.001	33.0	$y = 13 + 353.8x - 28.7x^2$	<0.001	57.4
MT4 strength	$y = 468.8 + 83.6x$	<0.001	50.2	$y = 75 + 287.0x - 20.8x^2$	<0.001	64.0
daily total phosphorus intake						
MT3 strength	$y = 431 + 60.5x$	<0.001	27.8	$y = -890 + 418.7x - 22.04x^2$	<0.001	52.0
MT4 strength	$y = 306 + 70.7x$	<0.001	45.2	$y = -673 + 336.8x - 16.36x^2$	<0.001	59.6

7.3.4 Bone mineralization

Effects of phosphorus level, phytase and the interactions on fat free weight, total phosphorus, percent phosphorus, total ash and percent ash in the fat free third and fourth metatarsals are shown in table 7.29. Treatment means are given in tables 7.30-7.34. Fat free weight of the metatarsals increased with phytase addition; in the fourth metatarsal this increase was seen at all except the highest level of phosphorus, whereas in the third metatarsal the increase was seen at levels of up to 3.25 g/kg digestible phosphorus. Total phosphorus in the bones was affected both by dietary phosphorus level and phytase (figure 7.10 and 7.11). Percent phosphorus in the bones was not particularly responsive to dietary phosphorus level, although it did decrease markedly in pigs receiving the basal level of phosphorus. Addition of phytase to diets containing up to 2.05 g/kg non-phytate-phosphorus resulted in an increased percent phosphorus of both the third and the fourth metatarsals.

Total ash and percent ash in the fourth metatarsal were affected by both dietary phosphorus level and phytase. Percent ash of the third metatarsal was not responsive to phytase, although total ash increased with enzyme addition at levels of up to 2.45g/kg digestible phosphorus.

Regression of bone mineral content against daily intake of non-phytate phosphorus yielded linear and quadratic relationships; these are presented in table 7.35. Relationships between bone mineral content and daily intake of total phosphorus are given in table 7.36. Increasing the intake of phosphorus yielded strongly significant ($p < 0.005$) increases in total phosphorus, total ash, and fat free weight of the third metatarsal, and in total phosphorus, total ash and percent ash of the fourth metatarsal.

Figure 7.10 **Effect of phosphorus level and phytase on total phosphorus of the third metatarsal**

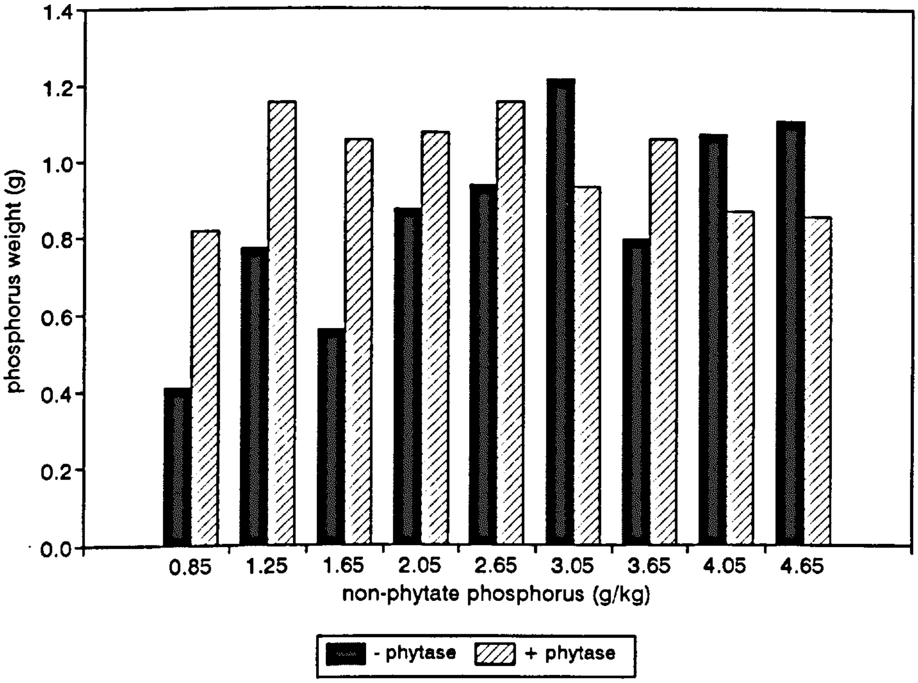


Figure 7.11 **Effect of phosphorus level and phytase on total phosphorus of the fourth metatarsal**

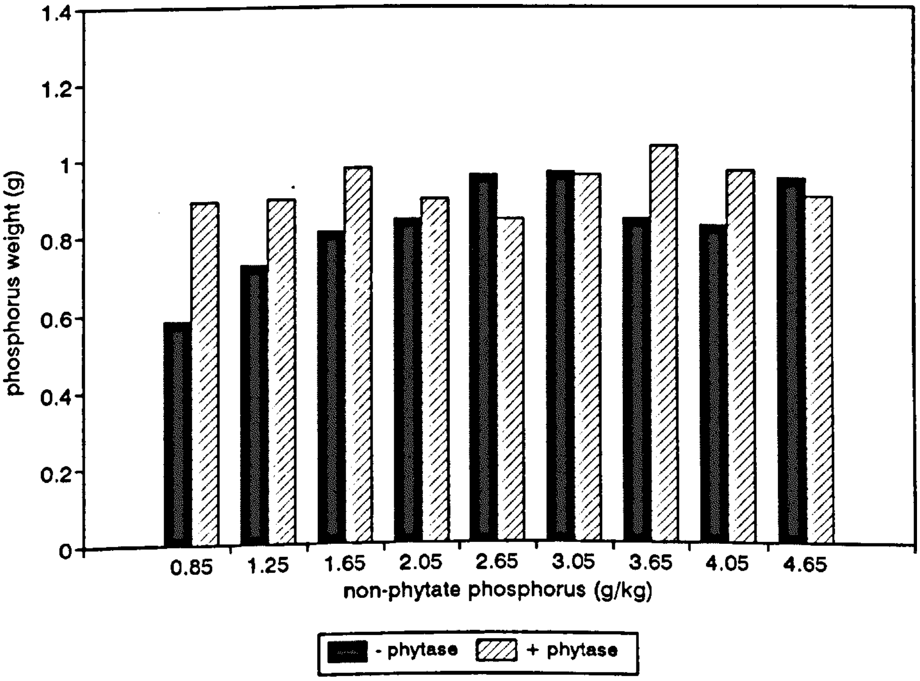


Table 7.29 Significance of effects of phosphorus level, phytase and the interactions on bone mineral content

	factor	p	sed
third metatarsal			
Fat free weight	P level * phytase	0.035	1.692
Total phosphorus	P level	0.003	0.110
	phytase	0.007	0.152
	P level * phytase	0.002	0.156
Percent phosphorus	phytase	0.086	0.360
Total ash	phytase	0.033	0.206
Percent ash	ns	>0.10	-
fourth metatarsal			
Fat free weight	phytase	0.061	0.435
Total phosphorus	P level	0.029	0.061
	phytase	0.001	0.029
	P level * phytase	0.030	0.086
Percent phosphorus	phytase	0.076	0.179
Total ash	P level	0.001	0.353
	phytase	0.001	0.166
Percent ash	P level	<0.001	1.251
	phytase	0.003	0.589

Table 7.30 Fat free weight of the metatarsals (g) - treatment group means

P level[r]	*3rd metatarsal*		*4th metatarsal*	
	- phytase	+ phytase	- phytase	+ phytase
0.85	14.27	16.09	15.42	16.95
1.25	16.98	17.91	14.42	16.54
1.65	13.29	18.01	16.50	16.94
2.05	15.20	18.28	17.30	17.32
2.45	16.87	19.53	16.19	17.82
2.85	18.16	15.01	16.73	17.17
3.25	16.34	17.01	18.39	18.82
3.65	17.75	16.04	16.49	18.11
4.05	17.84	16.11	17.78	17.04

Table 7.31 Total phosphorus of the metatarsals (g) - treatment group means

P level[r]	3rd metatarsal		4th metatarsal	
	- phytase	+ phytase	- phytase	+ phytase
0.85	0.373	0.810	0.592	0.893
1.25	0.757	1.166	0.723	0.902
1.65	0.564	1.065	0.811	0.980
2.05	0.870	1.076	0.836	0.896
2.45	0.930	1.160	0.952	0.838
2.85	1.218	0.932	0.963	0.954
3.25	0.805	1.075	0.831	1.032
3.65	1.074	0.876	0.817	0.964
4.05	1.092	0.842	0.955	0.904

[r] non-phytate phosphorus g/kg feed

Table 7.32 Percent phosphorus of the metatarsals - treatment group means

P level[r]	*3rd metatarsal*		*4th metatarsal*	
	- phytase	+ phytase	- phytase	+ phytase
0.85	2.58	5.23	3.84	5.28
1.25	4.33	6.49	5.05	5.48
1.65	5.50	5.97	5.02	5.78
2.05	5.85	5.97	4.74	5.18
2.45	5.47	5.95	5.87	4.82
2.85	6.71	6.40	5.76	5.55
3.25	4.95	6.47	4.53	5.49
3.65	6.10	5.58	5.10	5.31
4.05	6.18	5.27	5.41	5.33

Table 7.33 Total ash of the metatarsals - treatment group means

P level[r]	3rd metatarsal		4th metatarsal	
	- phytase	+ phytase	- phytase	+ phytase
0.85	3.430	4.617	3.314	4.651
1.25	4.607	5.236	4.027	4.988
1.65	4.504	5.234	5.014	5.148
2.05	4.687	6.173	4.688	5.428
2.45	5.056	5.561	4.873	5.435
2.85	5.598	4.838	4.963	4.948
3.25	4.718	5.458	5.346	6.155
3.65	5.265	5.026	4.965	5.347
4.05	5.081	4.773	5.218	5.404

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[r] non-phytate phosphorus g/kg feed

Table 7.34 Percent ash of the metatarsals - treatment group means

P level[r]	*3rd metatarsal*		*4th metatarsal*	
	- phytase	+ phytase	- phytase	+ phytase
0.85	23.35	29.20	21.42	27.46
1.25	26.53	29.51	28.02	30.20
1.65	38.03	29.17	30.45	30.39
2.05	31.42	34.18	27.18	31.37
2.45	29.59	28.51	30.06	30.52
2.85	30.80	32.76	29.68	28.77
3.25	28.89	33.31	29.07	32.59
3.65	29.70	31.14	30.29	29.51
4.05	29.40	29.56	29.51	31.39

[r] non-phytate phosphorus g/kg feed

Table 7.35 Regression of metatarsal mineral content (y) against daily intake of non-phytate phosphorus (x)

	Linear			*Quadratic*		
	equation of response	p	% var[τ]	equation of response	p	% var[τ]
Third metatarsal						
Fat free weight (FFW)	$y = 13.22 + 0.628x$	0.002	24.3	$y = 13.21 + 8.63x - 0.0003x^2$	0.008	21.9
Total phosphorus in FFW	$y = 0.45 + 0.086x$	0.001	28.3	$y = 0.19 + 0.215x - 0.013x^2$	0.002	29.3
Percent phosphorus in FFW	$y = 3.85 + 0.312x$	0.044	10.0	$y = 1.90 + 1.28x - 0.982x^2$	0.048	13.3
Total ash in FFW	$y = 3.73 + 3.728x$	0.004	20.8	$y = 3.30 + 0.439x - 0.023x^2$	0.013	19.5
Percent ash in FFW	-	ns	-	-	ns	-
Fourth metatarsal						
Fat free weight	$y = 17.76 + 0.387x$	0.014	14.7	$y = 14.13 + 0.712x - 0.033x^2$	0.047	12.6
Total phosphorus in FFW	$y = 0.68 + 0.031x$	0.021	13.4	$y = 0.40 + 0.178x - 0.015x^2$	0.002	29.5
Percent phosphorus in FFW	-	ns	-	$y = 3.05 + 0.903x - 0.841x^2$	0.041	13.7
Total ash in FFW	$y = 3.61 + 0.231x$	<0.001	35.7	$y = 2.76 + 0.667x - 0.045x^2$	<0.001	40.5
Percent ash in FFW	$y = 24.6 + 0.7732x$	0.003	21.7	$y = 20.14 + 3.11x - 0.239x^2$	0.001	30.7

[τ] percentage variance accounted for

Table 7.36 Regression of metatarsal mineral content (y) against daily intake of total phosphorus (x)

	Linear			*Quadratic*		
	equation of response	p	% var	equation of response	p	% var
Third metatarsal						
Fat free weight (FFW)	$y = 11.74 + 0.565x$	0.002	25.0	$y = 11.67 + 0.58x - 0.001x^2$	0.007	22.6
Total phosphorus in FFW	$y = 0.28 + 0.073x$	0.002	25.6	$y = -0.29 + 0.226x - 0.009x^2$	0.017	26.1
Percent phosphorus in FFW	$y = 3.31 + 0.256x$	0.063	8.1	$y = -1.05 + 1.419x - 0.071x^2$	0.075	10.6
Total ash in FFW	$y = 3.24 + 1.193x$	0.004	20.6	$y = 2.81 + 0.308x - 0.007x^2$	0.017	18.3
Percent ash in FFW	-	ns	-	-	ns	-
Fourth metatarsal						
Fat free weight	$y = 13.82 + 0.351x$	0.012	15.5	$y = 12.63 + 0.675x - 0.020x^2$	0.043	13.1
Total phosphorus in FFW	$y = 0.64 + 0.025x$	0.037	10.4	$y = -0.11 + 0.258x - 0.41x^2$	0.002	29.2
Percent phosphorus in FFW	-	ns	-	$y = 0.32 + 1.216x - 0.071x^2$	0.028	16.0
Total ash in FFW	$y = 3.18 + 0.202x$	<0.001	34.2	$y = 1.13 + 0.739x - 0.033x^2$	<0.001	38.5
Percent ash in FFW	$y = 23.23 + 0.649x$	0.006	19.1	$y = 12.04 + 3.68x - 0.188x^2$	0.002	28.3

τ percentage variance accounted for

7.3.6 Relationship between bone strength and bone mineral composition

Regression of strength against fat free weight and mineral content of the third metatarsal showed strong linear and quadratic relationships ($p < 0.001$) between third metatarsal strength and total ash, and between strength and total phosphorus. These were also apparent in the fourth metatarsal, which in addition showed a strong linear and quadratic relationship between strength and percent of ash in the fat free bone ($p < 0.001$). Addition of phytase weakened these relationships (table 7.37), except for the linear and curvilinear relationships between fourth metatarsal strength and total ash in the fat free bone, which were maintained. In addition, the original weak quadratic relationship between strength and fat free weight of the fourth metatarsal was strengthened by addition of phytase ($p=0.008$ vs $p= 0.018$).

7.3.7 Relationship between bone strength and growth

Regression of bone strength against daily liveweight gain showed linear relationships for both the third and the fourth metatarsals, although these were somewhat weak (MT3, $p= 0.036$; MT4, $p = 0.011$; table 7.38). Addition of phytase destroyed the relationship between third metatarsal strength and average daily gain, but maintained that between strength of the fourth metatarsal and average daily gain, although the relationship was not as strong as without the enzyme ($p = 0.036$).

7.3.8 Relationship between growth and carcase measurements

In the absence of phytase, there was a strong linear relationship between average daily gain and the width of the *longissimus dorsi* muscle (measurement "A"), which became non-significant when enzyme was added to the diet. However, a weaker relationship between average daily gain and the posterior *gluteus medius*, was strengthened by addition of phytase ($p= 0.06$ without phytase, $p < 0.001$ with phytase). In addition, phytase inclusion resulted in strongly significant ($p<0.001$) linear relationships between daily liveweight gain and P2, P3 and "C" measurements. Equations describing the responses are shown in table 7.39

7.3.9 Relationship between growth and bone mineral content

A strong linear relationship between total ash in the fourth metatarsal and average daily gain was revealed ($p= 0.001$) which was destroyed by phytase addition. Weaker responses of total ash in the third metatarsal, fat free weight and percent ash of the fourth metatarsal ($p< 0.01$) were also destroyed by enzyme addition. Equations describing the responses are presented in table 7.40

Table 7.37 Significance of the responses of metatarsal strength (y) to metatarsal mineral content (x)

	Linear		*Quadratic*	
	- phytase	+ phytase	- phytase	+ phytase
3rd metatarsal				
Fat free weight	0.018	ns	0.063	0.084
Total ash	<0.001	0.050	<0.001	ns
Percent ash	ns	0.044	0.032	0.047
Total phosphorus	<0.001	0.012	<0.001	0.036
Percent phosphorus	0.017	0.025	0.032	0.080
4th metatarsal				
Fat free weight	0.004	0.002	0.018	0.008
Total ash	<0.001	<0.001	<0.001	<0.001
Percent ash	<0.001	0.011	<0.001	0.002
Total phosphorus	<0.001	0.030	<0.001	0.012
Percent phosphorus	0.013	ns	0.034	ns

Table 7.38 Significance of the responses of metatarsal strength (y) to daily liveweight gain (x)

	- phytase			+ phytase		
	response equation	p	% var[r]	response equation	p	% var[r]
3rd metatarsal	$y = 389 + 730x$	0.036	10.3	-	ns	-
4th metatarsal	$y = 279 + 824x$	0.011	16.3	$y = 448 + 758x$	0.039	9.9

[r] percentage variance accounted for

Table 7.39 Significance of the responses of carcase measurements (y) to daily liveweight gain (x)

	equation of response	p	% var[τ]
- phytase			
Posterior *gluteus medius*	$y = 4.37 + 8.23x$	0.054	8.6
P1	$y = 3.56 + 4.12x$	0.06	7.8
Width of *longissimus dorsi* (a)	$y = 95.12 - 16.46x$	0.002	23.7
+ phytase			
Maximum shoulder	$y = 19.72 + 8.81x$	0.063	7.6
Minimum mid-back	$y = -0.02 + 11.36x$	0.002	24
P1	$y = -0.84 + 10.10x$	<0.001	39.6
P2	$y = - 0.67 + 9.12x$	<0.001	35.8
P3	$y = -0.15 + 9.36x$	<0.001	26.9
c	$y = 0.25 + 8.01x$	<0.001	29.5

Table 7.40 Significance of the responses of bone mineral content (y) to daily liveweight gain (x)

	- phytase			+ phytase	
	response equation	p	%var[τ]	p	%var[τ]
3rd metatarsal					
Total ash	$y = 2.85 + 2.69x$	0.034	10.6	ns	-
4th metatarsal					
Fat free weight	$y = 12.76 + 5.39x$	0.039	9.9	ns	-
Total ash	$y = 2.39 + 3.26x$	0.001	26	ns	-
Percent ash	$y = 20.53 + 10.99x$	0.011	15.9	ns	-

[τ] percentage variance accounted for

7.4 Discussion

7.4.1 Growth performance

Although at first it appeared that responses of growth parameters to phosphorus and phytase were non-significant, re-examination of the differences between pigs fed with and without phytase at discrete levels of phosphorus revealed significant effects. Analysis of variance calculates an average significance across treatments. In cases where differences are mostly non-significant, as in the present experiment, individual significant differences may be over-ridden by the average "p" value. The power of the analysis of variance design for the present experiment was therefore limited, but served to indicate the response to phytase at each level of dietary phosphorus.

Regression of daily liveweight gain against daily intake of phosphorus showed both linear and curvilinear responses. However, maximum daily gain was achieved only at the greatest phosphorus intake within the range used. Thus it was presumed that within the range of phosphorus intakes seen, the response was linear. Beyond this range, it would be expected that growth followed either a broken line or a quadratic model, as growth would not increase indefinitely in response to phosphorus intake.

The foremost question raised from the response of growth to phosphorus intake was whether the response seen was simply due to increasing feed intake, or whether it was a response to phosphorus intake *per se*. It may be speculated that by increasing feed intake, phosphorus intake increases, thus the response seen was mainly due to potentially larger pigs consuming more feed and growing at a faster rate. However, no significant relationships were seen from the regression of feed intake against phosphorus intake. Thus it may be proposed that phosphorus content of the feed was influencing growth. Examination of the feed intake data revealed depressed feed intake at the lowest levels of dietary phosphorus, which although non-significant, were nevertheless pronounced, and it can be reasonably assumed that phosphorus level was influencing feed intake which in turn influenced growth of the pigs.

Due to the linearity of the growth response, a phosphorus requirement for maximum growth could not be established. Pigs growing at the greatest rates consumed the most food, thus there may have been another limiting constraint besides phosphorus. Had the phosphorus level of the diet been raised an increased phosphorus intake may have resulted. Nonetheless, it could be seen that the maximum growth rate obtained (890 g/day) required a daily intake of 9g digestible phosphorus. Based on the mean daily feed intake, a level of 4.65g/kg digestible phosphorus would be sufficient to support growth; this was somewhat higher than previous recommendations of the ARC (1981) and much higher than the recent estimates of Jongbloed (1992) who proposed that a level of 2.3g/kg digestible phosphorus for pigs of 30kg liveweight could be further reduced to 2g/kg at 50kg liveweight. Latter estimates were obtained using factorial data from slaughter experiments, and assume a growth rate of 780g/day, from 30-110kg. Based on the regression equation obtained from the present trial, a dietary allowance of 2.3g/kg phosphorus would support a daily growth of only 612g, whereas mean growth rate of pigs in the current trial was 720g/day. However, caution must be exercised when comparing the data, as a narrower band of growth was used in the present experiment. Table 7.41 compares present results with current recommended phosphorus levels for growing pigs. A comprehensive comparison of present with published data was precluded because many previous authors had used total, rather than digestible phosphorus when estimating requirements.

In comparing the linear response of growth ($y = 0.548 + 0.041x$) to that obtained in the previous trial ($y = 0.518 + 0.22x$) it could be seen that in order to support the same growth, more phosphorus was needed for growing pigs (25-60kg liveweight) than for young pigs (less than 25kg liveweight). Phosphorus absorption and retention, as a percentage of total phosphorus intake, has been found to decrease linearly with increasing liveweight (Kirchgessner *et al.*, 1963; Morgan *et al.*, 1969) although whether this is due to an effect of physiological age, or to a surplus in the amount of phosphorus offered in relation to requirement has been subject to debate. Since the present experiment used a range of phosphorus intakes, starting below requirement, the results suggest a physiological effect; ie older pigs may not be as efficient at absorbing and/or utilising phosphorus.

Table 7.41 **Comparison of results with current recommended phosphorus levels for growing pigs**

Source	Liveweight (kg)	DLWG (kg)	Daily P (g)
Present results	25-60	0.89	9.0 digestible
ARC (1981)	25	0.55	6.1 total
	45		7.4 total
NRC (1988)	20-50	0.70	9.5 total
Gueguen and Perez (1981)	35	0.60	9.5 total
	50	0.75	11 total
Jongbloed *et al.*, (1993)	30	0.57	3.12 total
	50	0.82	4.40 total

While the depressive effect of low phosphorus on feed intake is recognised, and specific appetites have been demonstrated, particularly in cattle (Fishwick *et al.*, 1977; Bass *et al.*, 1981) and in poultry (Holcombe *et al.*, 1976), physiological mechanisms have not been elucidated. So far, few data relate to pigs, but present results indicated that very low intakes of phosphorus certainly depressed appetite. Reduced daily liveweight gains at low levels of phosphorus were overcome by addition of phytase to the diet. This could be attributed mainly to an increased feed intake. Dietary enzymes are generally associated with an increased feed intake, but in the present case it appeared that the increased feed intake occurred as a result of increased available phosphorus, rather than due to the enzyme preparation *per se*, as increased feed intake with the enzyme was not seen at higher levels of phosphorus.

Feed conversion efficiency responded quadratically to increasing phosphorus intake. Optimum efficiency was achieved with a daily intake of 6g digestible phosphorus, thereafter growth became less efficient. At very low (0.85 g/kg) and very high (4.05 g/kg) dietary levels of phosphorus, efficiency was poor, probably due to the calcium to phosphorus ratio being too high (at 3.2) and too low (at 1.4), respectively.

Phosphorus levels used in the present experiment were lower than those of the previous experiment as it was anticipated that the requirements for pigs of this size would be lower than that of very young pigs. However, in view of the daily gain response, it may have been appropriate to use a range that reached more than 4.05g/kg digestible phosphorus. The amount of digestible phosphorus required for optimum feed conversion efficiency calculated from the present experiment, at 6g day was higher than the net requirement of the ARC proposed in 1981.

The suggestion that requirements for optimum feed efficiency are less than those for maximum growth is in accordance with results of Combs *et al.* (1991) which showed that while 100% of NRC (1979) requirements were necessary for maximum growth, 83.5% was sufficient for optimum feed conversion efficiency. In another experiment, increased dietary calcium and phosphorus resulted in linear improvements in gain and feed intake, although feed conversion efficiency was unaffected (Maxson and Mahan, 1983). Others have found no effect of phosphorus level on growth performance, but in many of these experiments (Brennan and Aherne, 1986; Nimmo *et al.*, 1980; Donzele *et al.*, 1988), diets were not formulated to be deficient in phosphorus, but remained above NRC requirements. Ketaren *et al.* (1993) observed a quadratic response of feed intake and growth rate to available phosphorus level in pigs growing from 20 to 90kg liveweight. Maximum growth rate (955 g/day) and feed intake (2118 g/day) were achieved 3g/kg digestible phosphorus.

The quadratic response of feed conversion efficiency obtained suggested that efficiency of growth altered with phosphorus intake. The metabolic basis for this change in efficiency is not known. When genetically improved pigs were fed low calcium and phosphorus levels the amount of metabolisable energy required for liveweight gain was increased from 25.7 MJ/kg to 27.8 MJ/kg (K'nev *et al.*, 1987). Ludke *et al.* (1989) observed that fat retention in soft tissues and the skeleton increased by 0.7kg when phosphorus was lowered from 5.47g/kg to 2.97, while protein retention decreased by 1.5kg, although these authors concluded that energy utilisation for growth was not influenced by phosphorus supply. In another experiment it was found that nitrogen balance was not affected by dietary calcium and phosphorus (Donzele *et al.*, 1988).

7.4.2 Carcase measurements

Pigs were slaughtered at 60kg because it was anticipated that requirement for growth would start to change beyond approximately 60kg, as the pig approached the "finishing" stage of growth. While few relationships between daily phosphorus intake were seen in the absence of phytase, when the enzyme was added to the diet, some (weak) relationships became apparent (P1, P2, A, C). These were difficult to explain, particularly in light of the results of the previous experiment, where addition of phytase destroyed relationships between phosphorus intake and growth, carcase mineral content, bone breaking strength and bone mineral content.

Of approximately 20% of total body phosphorus located in the soft tissues, most is present in muscle, thus the lack of response of lean tissue growth to phosphorus intake was surprising. Zivkovic *et al.* (1991) observed that daily gain improved in response to increasing phosphorus intake, but carcase yield was unaffected. In another trial (Lorek *et al.*, 1995), physical and chemical properties of meat and fat from pigs fed either 0.4 or 0.6% phosphorus were unaffected, but the chemical composition of lean differed, with the lower phosphorus level yielding a lower crude protein and higher fat than the higher phosphorus level (23.2 vs 21.9% protein; 2.21 vs 3.24% fat). The genetic "type" of pig may also influence responses to phosphorus. Fat- type pigs retained more phosphorus than lean genotypes, although no differences in carcase yield were observed (Ivanchuk and Mal'tseva, 1986). Addition of phytase led to the establishment of increased backfat (P1,P2) with increasing phosphorus intake, suggesting that perhaps growth was not as efficient in pigs receiving the enzyme.

While the mineral concentrations of pigs in the present experiment were not measured, it would be reasonable to suggest on the basis of the present experiment, that increases in whole body mineral in response to phosphorus seen in the previous experiment, were due to increases in bone mineralization rather than to higher concentrations of phosphorus in the soft tissue. On the other hand, concentrations of calcium and phosphorus are higher in the adult than in the newborn (Georgievskii, 1981), and the relative proportion of protein to ash changes from 3:1 in the neonate to 5:1 in older pigs (Shields Jr, 1991), these may have been confounded with responses to phosphorus and phytase.

7.4.3 Physical properties of the metatarsals

A quadratic response of metatarsal strength to increasing phosphorus intake was assumed on the basis that as phosphorus intake increases above requirement, the changing Ca:P ratio becomes unfavourable for net bone deposition. Based on the surmise that bone strength increases linearly within the range of phosphorus levels used typically during a slope-ratio assay, it was inferred that the linear response seen was a manifestation of the ascending region of a quadratic curve. The model agreed with that obtained by Combs *et al.* (1991) when 75-130% of NRC recommended phosphorus levels were fed. In contrast, Cromwell *et al.* (1993) and Ketaren *et al.* (1992) found linear responses at up to 4g/kg and 3 g/kg phosphorus, respectively.

While a daily intake of 5.5g digestible phosphorus was sufficient for maximum strength of the third metatarsal, 6.5g was necessary for maximum strength of the fourth. The latter is the outer bone in the foot, and being larger than the inner third metatarsal, requires greater mineralization for maximum strength.

It was possible that the increased bone strength seen was due to increased size alone, with no effect of mineralization. Mudd *et al.* (1969) found that calcium intakes above requirement induced larger bone mass, but not an increased mineralization, and it is possible that this may also hold true for phosphorus. Mineralization *per se* was not measured (for example using bone densitometric x-ray techniques), although chemical analysis was carried out. In addition, the width and breadth of the bones was measured at the (approximate) mid-shaft, but these data were ignored as it was almost impossible to take consistent measurements exactly in the centre of the shaft, due to the bones' very irregular nature. However, freshweight of the bones was recorded, and no direct effect of phosphorus level was seen.

The present experiment used metatarsals, rather than femurs, due to practical and economic constraints for pigs of this size. Despite the lack of response of these bones in the previous trial, it was assumed that pigs of this age, in having a more developed skeleton, would be more responsive to phosphorus level, and thus also to phytase.

Addition of phytase resulted in an increased breaking strength of both the third and the fourth metatarsals, due to release of phytate-phosphorus which was then utilised for bone accretion. At higher phosphorus levels this increased strength was not seen because there was already enough phosphorus present in the diet to support bone development. Responses to the enzyme obtained from the present trial were more pronounced than those of Cromwell *et al.* (1993), where decreased bone strength of pigs receiving low-phosphorus diets was only partially restored by phytase.

Based on the response of the metatarsals to phytase, it was calculated that 50-70% of phytate-phosphorus was converted to a digestible form by the addition of phytase (table 7.42), which was lower than that calculated from the previous experiment, although higher than the 30% conversion obtained by Cromwell *et al.* (1993).

7.4.4 Mineralization of the metatarsals

Of the criteria studied, total phosphorus and total ash appeared to be the most sensitive to phosphorus and phytase. Calcium content of the bones was not measured since no significant responses were seen in the previous method. Mineralization could similarly have been measured using alternative methodology such as photon absorptiometry or radio-isotope kinetics, but present results suggested that analysis of mineral content, combined with mechanical measurement of break strength, were adequate descriptors.

Mineral deposition is the net result of accretion and bone resorption, the latter being particularly important for bone modelling. Fernandez (1995) found no difference in accretion with increasing dietary phosphorus, however, a decrease in bone resorption was observed. In fact, intestinal absorption accounted for 86% of the variation in bone resorption. Thus, it could be presumed that uptake of dietary phosphate was a major determinant of bone mineralization.

Although the metatarsals had been dismissed as unsuitable indicators of phosphorus availability in very young pigs, it was presumed that in older pigs, having a more mature skeletal systems, the bones would be sufficiently well-developed to show sensitivity to phosphorus intake. Whether or not these bones were indicative of the skeleton as a whole was questionable. Fernandez (1995) found that metacarpals and femurs had similar accretion rates. On the other hand, ash content of the coxae, tibia and fibula, and radius and ulna were more responsive to dietary phosphorus than other bones in pigs of 20-50kg (Ketaren *et al.*, 1993). Furthermore, it has been established that during deficiency, withdrawal of phosphorus occurs first from the spongy bones (ribs, vertebrae and sternum). The shafts of the long bones and the small bones of the extremities affected last (Underwood, 1966). However, in the present experiment the metatarsals were sufficiently sensitive to availability and were useful as indicators of bone growth. Furthermore, they were relatively easy to obtain without devaluating the carcase. Metatarsals of the hind foot, rather than the metacarpals of the fore were used as the former were found to be more sensitive to phosphorus intake (Lepine *et al.*, 1985).

The quadratic response of bone mineralization with increasing phosphorus was somewhat difficult to justify. It seemed more likely that mineralization would plateau and increase no further, rather than decline, in response to increasing phosphorus intake. Consideration of homeostatic mechanisms and the inter-relationship between calcium and phosphorus provided a possible explanation of the declining mineralization. As phosphorus intake exceeded requirement, calcium-to-phosphorus ratio of the blood decreased, stimulating parathyroid hormone, which promoted resorption of bone from the skeleton into the blood. Data of Ketaren *et al.* (1993) support this theory. Bone mineralization was found to increase linearly in response to dietary phosphorus level when the calcium-to- phosphorus ratio was kept constant, but when the ratio was variable, a quadratic response was obtained. Similar quadratic responses of bone ash to dietary phosphorus of growing pigs were observed by Maxson and Mahan (1983).

Accepting a quadratic response for mineralization, it was calculated that maximum mineralization of the third metatarsal was achieved with a daily intake of 8.5g digestible phosphorus, whereas maximum mineralization of the fourth metatarsal required only 6g/day. The latter result was surprising as maximum strength for this bone was achieved with an intake of 6.5g/day. It would be expected that maximum strength would be reached at the same point or in advance of maximum mineralization, since the latter is required to provide strength. The result was also unexpected in view of the presumption that the fourth metatarsal, being larger than the third, required more phosphorus for development. It has been established that mineral deposition continues even after maximum strength has been reached (Crenshaw, 1981) and although this was seen with the third metatarsals, results of the fourth metatarsals, could not be explained in light of current knowledge, except by inaccuracy of the regression equation. The latter explanation was confirmed by calculating the daily phosphorus requirement for maximum ash of the same bone, which, at 7.5g, was closer to the expected value for maximum mineralization. Results disagreed with the prediction of Crenshaw (1986) that breaking force of the third metatarsal could be reliably predicted by mineral content ($r^2 = 0.90$). Using percent bone ash as the criterion of response, Ketaren *et al.* (1993) proposed a dietary level of 0.3% available phosphorus for bone development of growing pigs. However, current results suggested that bone ash, while giving a good indication of phosphorus availability, was not a suitable criterion on which to base requirement, as maximum mineralization did not correspond to maximum bone strength.

Increased fat-free weight of the metatarsals when phytase was added to the diet may have been partly due to increased growth of the pigs, resulting in larger bones. However, percent ash and phosphorus of the bones also increased in response to phytase, confirming that the increased bone weight was at least partially due to increased mineralization.

Factors other than dietary phosphorus intake are reflected in bone mineral content and mechanical properties, and must therefore be considered when comparing experimental data. *Ad libitum*, as opposed to restricted feeding resulted in larger, heavier, stronger bones in boars (Lepine *et al.*, 1985). To avoid gender differences in response to phosphorus and phytase which had been noticed in the previous trial, all males were used in the present trial. This gave greater statistical power than in the previous trial; on the other hand the requirements estimated from responses of boars were not necessarily indicative of those of a mixed gender population.

7.4.5 Comparison of response criteria

Probability values, percent of variance accounted for by the regression equations, and phosphorus intake required to obtain maximum response of the various criteria are compared in table 7.43. Results confirmed that growth, when regressed against phosphorus intake rather than dietary phosphorus level, was a sensitive criterion of response. In contrast, carcase traits were influenced little by phosphorus intake. Bone strength was more sensitive to phosphorus availability than bone mineral content, although the latter was extremely useful as an indicator of bone development. Present results confirmed those of the previous trial where requirements for bone strength were less than those for growth.

7.4.6 Phosphorus requirement of growing pigs

Based on the mean feed intake of 1936 g/day, dietary phosphorus levels needed to support maximum response of the various criteria were calculated. These are compared with published data in table 7.44. The requirement for growth, at 4.65g/kg digestible phosphorus, was somewhat higher than previous recommendations (ARC, 1981; NRC, 1988; Jongbloed *et al.*, 1992). Maximum feed conversion efficiency was obtained with a lower phosphorus level of 3.1g/kg. Based on the mean requirement for strength of the third and fourth metatarsals, a level of 3.1g was also calculated for maximum bone strength. This level was much nearer requirements for growth and bone development proposed by Ketaren *et al.* (1993) and the ARC (1981). The actual dietary requirement will depend on whether fast growth, or efficient growth is of primary importance. Early growth of the boar is critical and a sound mineral regime necessary to ensure maximum bone and muscle development because of rapid mineral deposition rates in early life (Nimmo *et al.*, 1980). In the case of breeding stock, maximum bone mineralization, rather than maximum bone strength, may be more critical, as a store of phosphorus during lactational demands, and in these cases the allowance would tend towards the higher requirement.

7.4.7 Phosphorus/phytase equivalence

It was apparent from measurements of growth and bone strength that differences in performance achieved with phytase diminished as the dietary phosphorus level increased. Although establishment of regression responses both with and without phytase was intended, by using a constant level of phytate-phosphorus, addition of the enzyme destroyed all linear and curvilinear responses of bone strength to phosphorus intake. This was similar to results of the previous experiment, and again suggested either some disruption of the homeostatic control mechanism of pigs receiving phytase, or more possibly, an interaction of inorganic phosphorus with phytase. The net result would be that different amounts of phosphorus were made digestible, which would subsequently be reflected in bone development. Despite these anomalies, by comparing response criteria at the basal level of phosphorus with and without the enzyme, it could be seen that approximately 50% of phytate phosphorus was being made available for digestion (table 7.42), and this estimate of was consistent whether based on growth, bone strength or mineralization.

7.5 Conclusions

Daily requirement of digestible phosphorus were estimated at 6g for optimum feed conversion efficiency, 9g to support a growth rate of 0.89 kg/day, and between 5.5 and 6.5g for maximum bone strength.

Addition of phytase enabled approximately 50% of the phytate-phosphorus to be utilised. This estimate of phosphorus/ phytase equivalence was consistent across criteria, although differences in performance achieved with phytase diminished as dietary phosphorus level increased.

Table 7.42 Calculation of phytate-phosphorus made available by phytase

Non-phytate-P in basal diet = 0.85 g/kg
Total P in basal diet = 2.5 g/kg
Phytate P (N-P-P) = 1.65 g/kg

DLWG at 0.85g/kg N-P-P (+ phytase) ≡ DLWG at 1.65g/kg N-P-P (-phytase)
MT3 strength " " ≡ strength at 2.05g/kg N-P-P (-phytase)
MT4 strength " " ≡ strength at 1.65g/kg N-P-P (-phytase)
MT3 P " " ≡ MT3 P at 1.65g/kg N-P-P (-phytase)
MT4 P " " ≡ MT4 P at 2.05g/kg N-P-P (-phytase)

Assuming that all extra phosphorus has become available through the use of phytase, Phosphorus made available through phytase (lower and upper limits):

= 1.65 - 0.85 2.05 - 0.85
= 0.80g/kg = 1.20g/kg

As proportion of phytate phosphorus = (0.80/1.65) * 100 (1.20/1.65)*100
= 48% = 72%

Table 7.43 Goodness of fit of regressions, and phosphorus required for maximum response of various criteria

Criterion	Shape of response	P	% var	P required for max. response (g non-phytate P/day)
DLWG	linear	<0.001	52	≥9
FCE	quadratic	0.009	21.7	6
MT3 strength	quadratic	<0.001	57.4	5.5
MT3 ash	quadratic	0.002	29.3	7.0
MT3 phosphorus	quadratic	0.002	29.3	8.5
MT4 strength	quadratic	<0.001	64	6.5
MT4 ash	quadratic	0.013	19.5	7.5
MT4 phosphorus	quadratic	0.002	29.5	6.0

Table 7.44 Comparison of phosphorus requirements for growing pigs with published data

Source of recommendation	Liveweight (kg)	P (g/kg FW)
Present results:		
Maximum growth	25-60	4.65 digestible
Optimum FCE	25-60	3.10 digestible
Bone strength	25-60	3.10 digestible
ARC (1981) - growth and bone strength	25-45	5.1-3.7 total[r]
NRC (1988) - growth	20-50	2.5 digestible
Gueguen and Perez (1981) - growth	30-50	6.0-5.6 total[r]
Jongbloed (1992) - growth	30-50	2.3-2.0 digestible[r]
Ketaren *et al.* (1993) - growth and bone strength	20-90	3.0 digestible

Chapter 8 Concluding discussion

8.1 Phosphorus requirement and availability

Although the mechanism by which phosphorus influences appetite remains unknown, a definite association between phosphorus level in the feed and feed intake was demonstrated by the data of the growth trials (*Sections 6.3* and *7.3*), which showed that phosphorus deficiency reduces appetite. In young pigs (10-25kg liveweight) a gradual reduction in feed intake was observed as the digestible phosphorus content was lowered from 4 to 2g/kg, and in pigs growing from 25-60kg liveweight, feed intake decreased as digestible phosphorus was lowered from 2.45 to 0.85g/kg.

Increases in feed intake largely accounted for the increases in liveweight gain achieved as phosphorus intake was increased and suggested no effect of phosphorus on growth above that of stimulating appetite. Despite this, recent work (Whipp, 1994) demonstrated that phosphorus retention rate increases with growth rates (figure 8.1), thus faster growing pigs require more phosphorus which, if not provided, will result in reduced feed intake and growth depression. This is particularly important when considering that modern breeding practises, while improving growth rates, have done little to increase appetite. Thus, correct phosphorus intake is particularly critical to prevent feed intake reduction in modern "fast growing" hybrids, which, by definition grow at rates of more than 1kg per day during the growing-finishing period.

The lack of response of carcase composition to phosphorus intake suggested no effect of phosphorus on the relative deposition rates of lean and fat tissue. This was somewhat surprising since phosphorus is an important component of both muscle and fat. While fat to lean ratio may not be affected, there may be an influence of phosphorus intake on chemical composition of the lean tissue. Analysis of the chemical composition of lean showed an effect of increasing phosphorus from 4 to 6g/kg in pigs of 20-110kg (Lorek *et al.*, 1985). Higher levels of crude protein and lower levels of fat were observed, while carcase quality and physico-chemical properties of lean and fat were similar. In the present study, in view of the lack of response of carcase composition in the present study, it was presumed that the marked effect of phosphorus intake on phosphorus content of the whole body was probably mainly due to increased mineralization of the skeleton.

Although changes in phosphorus retention with growth were not studied, published data agreed that in the whole animal, phosphorus concentration of the body decreases with age, while per kg gain, phosphorus retention remains constant (estimated at 5.6g P/kg liveweight; Jongbloed, 1987). This apparent contradiction may be due to the decreasing proportion of bone dry matter to the total body dry matter with ageing (Maxson and Mahan, 1983), and suggests a declining requirement as the pig matures.

The growth trials showed an increased requirement per kg liveweight growth in older pigs (25-60kg) compared with younger pigs (10-25kg) which may have been due to less efficient retention as the pig matured. Factors which may have accounted for this (ie absorption or subsequent utilization of phosphorus) were not accounted for in the current study; it was assumed that all dietary inorganic phosphorus was absorbed and utilized if a requirement existed. These assumptions may have been over-simplifications. On the other hand, while the balance studies showed a decreasing dietary concentartion required with age, this was due to increased feed intake providing an increased total phosphorus intake, rather than a lower net requirement. The progression of phosphorus retention with growth is complex and may be a function of physiological age (ie nearness of the animal to maturity) rather than liveweight *per se*. While it has so far been accepted that efficiency of phosphorus absorption decreases with age, this assumption has been challenged by recent work (Fernandez, 1995). Most work so far has used a "piecemeal" approach using isolated components of phosphorus metabolism (eg absorption); which may not represent the situation *in vivo*. Further work using a modelling approach, is clearly needed.

The pig has a skeleton which supports a greater weight in relation to body size than any other farm animal. In accepting that the requirement for growth is less than that for maximum bone strength, assessment of the bone strength required to withstand the forces imposed upon the pig at various stages during growth could be argued as being a fundamental step in quantifying the phosphorus requirement. However, results of the growth trials (*Sections 6.3* and *7.3*) indicated that, in pigs growing up to 60kg liveweight, by feeding a level of phosphorus which allows maximum growth, bone strength may already be achieved. The marked discrepancy between this and most previous work may have been due to pigs in the present being in the early stages of growth. In later finishing stages, the current observation may not hold.

The third and fourth metatarsals were responsive to phosphorus intake in pigs growing from 25-60kg liveweight, while in younger pigs (10-25kg liveweight) the femurs showed a better response. Despite marked increases in metatarsal breaking force in pigs of 25-60kg in response to increased phosphorus, relatively small differences in ash content were observed, suggested that increased bone mass, rather than mineralization, caused the observed increases in strength. Breaking strength determination using the Instron tester may therefore be more useful than mineral analysis in calculating phosphorus requirements, as the present work showed that maximum mineralization did not necessarily indicate maximum bone strength. Furthermore, Crenshaw (1986) proposed that effects of phosphorus deficiency may be detected by mechanical test before changes in mineral content are detectable.

Conflicting opinions existed as to whether empirical or factorial techniques were most suitable for assessing phosphorus requirements. While factorial assessment was claimed to be scientifically the most valid technique (Gueguen and Perez, 1981), being applicable to all metabolic states, the empirical approach allowed nutrient interactions to be considered, and was recommended for estimating phosphorus retention (Walz and Pallauf, 1991) as it indicated the course of retention during growth. Use of serial slaughter would have allowed a factorial approach to taken, but, by constraining the number of animals available per treatment, would have interfered with the original objectives of looking at the effect of phytase on phosphorus availability. Thus the empirical approach was adopted in this study, using balance trials, carcase analysis and bone parameters as criteria of response. Differences in estimates of phosphorus requirement and availability were found, depending on the criterion of response. In young pigs, phosphorus requirement for maximum growth performance and maximum mineral retention in the whole body was higher than that for maximum bone strength and mineralization. Published work showed similar discrepancies. For example, Ketaren *et al.* (1993) showed that calculations of phosphorus retention by balance were 11% higher than results by carcase analysis. Thus, a single criterion may not be sufficient to quantify mineral requirements, and an understanding of the partitioning and homeostasis of phosphorus may be more useful. While it has so far been assumed that growth and feed conversion efficiency were not sensitive to phosphorus intake, it was shown that if regressed against daily phosphorus intake, rather than dietary phosphorus level, these were suitable parameters from which to calculate phosphorus requirements (*Sections 3.3* and *4.3*). This was important, since growth and feed conversion efficiency are certainly the criteria with the most economic impact.

A retention of approximately 4g phosphorus/day for growing pigs was calculated from the balance experiments. However, growth of these pigs was slowed by their being confined in metabolism crates, and it was presumed that phosphorus requirements were lowered. Calculations based on growing pigs in the growth trial estimated digestible phosphorus requirements to be 6 g/day for optimum feed conversion efficiency and maximum bone strength, but at least 9g/day for maximum growth. In young animals (10-25kg liveweight), maximum growth required 5.5g/day of digestible phosphorus, while 4g/day was sufficient for maximum bone strength. A comparison between present calculated phosphorus requirements and those from other recent works is made in table 8.1. It was recognised that net phosphorus requirement altered as a function of feeding level, performance, housing, genotype and growth rate, and that a single "universal" recommendation was inappropriate.

Dietary phosphorus recommendations in the literature differed widely, due to variation in availability of phosphorus in feed ingredients. Recommendations were based on calculated availability values for individual ingredients, which, due to experimental techniques, were subject to error. It may be practical to assume that the non-phytate portion of dietary phosphorus is digestible, and to take into account the availability of phosphate contained in mineral supplements by solubility tests, rather than to ascribe availability values for each feed ingredient. Practical formulation of dietary phosphorus levels based on these values may be particularly appropriate if the use of phytase is to be considered. In the present study, values of phytate-phosphorus were taken from published tables, but the use of near infra red reflection spectroscopy has also been used to determine phytate levels in feedstuffs (de Boever *et al*, 1994) and may be a useful tool for future formulation based on non-phytate phosphorus.

Formulation in this way obviates the need to determine phosphorus availability of individual ingredients. Instead, recommendations based directly on net phosphorus requirements become appropriate. However, differences in feed intake between pigs must not be ignored. While the "safety margin" could be lowered from the current 10% to around 5% on the basis of increased accuracy of dietary recommendations, it should not be removed completely. Much of the recent published which suggests a lowering of dietary phosphorus is based on an intake "just sufficient" to maintain performance, which, under commercial conditions, is not acceptable.

Refinement of the calcium to phosphorus ratio to accommodate the recommendation of using digestible phosphorus, may be appropriate but it should be realised that if phytase is to be included into the diet, the final calcium to digestible phosphorus ratio in the intestine will be lowered. Furthermore, feed compounders currently tend to put too much calcium in diets, which may result in reduced phytate hydrolysis, due to the formation of insoluble calcium-phytate (Williams and Taylor, 1985).

While attention was given to the effects of dietary phosphorus on calcium availability because of the close physiological inter-relationship between calcium and phosphorus, the experimental diets were designed to keep levels of all nutrients except phosphorus constant and non-limiting. Thus the results yielded little information about the effects of phosphorus and phytase on calcium digestion and utilisation.

8.2 Efficacy of *Aspergillus niger* phytase

Data from the literature and the present experiments established a repeated effect of *Aspergillus niger* phytase on apparent phosphorus digestibility by hydrolysis of phytates to release inorganic phosphorus, which was then absorbed. The extent of phytate degradation was dependent on the level of phytase and the nature of the diet.

Using a high-phytate diet with little or no supplemental inorganic phosphate allowed up to 70% phytate-phosphorus to be utilised. Release and subsequent utilisation of phytate-phosphorus allowed animals receiving phosphorus-deficient diets to achieve similar growth performances to those receiving diets adequate in phosphorus.

Levels of 500-1500 units phytase activity/kg feed were typically used in published experiments, one unit being defined as the amount of enzyme that will liberate 1μmol of orthophosphate from 1.5 mmol sodium phytate within 1 minute at 37°C and pH 5.5. While maximum increases in phosphorus digestibility were achieved with a level of 1000 units/kg, a considerable response to 400-500 units/kg suggested that this level may be the economic optimum. A quadratic response of phosphorus digestibility to phytase level was seen, which suggested a cumulative limiting response. The literature verified competitive end-product inhibition of plant phytase, and the diminishing effect of phytase as dietary phosphorus increased in the growth trials suggested that this mechanism may be significant for *Aspergillus* phytase. Research into the interaction between phytase and inorganic phosphorus should be given priority as it affects the way in which phytase can be used. It may be inappropriate to use inorganic phosphate supplements concurrently with phytase.

The assay procedure for phytase was consistent across experiments, in contrast to that of other exogenous enzymes for which variation in assay procedures according to enzyme source is a contentious issues. No evidence was found to support the suggestions that HPLC analysis should be used to quantify the intermediate products of phytase degradation, although this may be useful as a tool to develop of a "potent" phytase, that is, one which hydrolyses phytate completely to inorganic phosphate.

While repeated effects of phytase on phytate digestibility has been shown in pigs, results using other exogenous enzymes show inconsistent responses. Thus it is often assumed that the nature of the gastrointestinal tract in pigs is unsuited to exogenous enzyme use. Based on the successful use of phytase it would be useful to study in more detail the action of other enzymes within the gastro-intestinal tract of the pig.

Effects of phytase on phosphorus availability were consistent, however effects on the digestibility of other nutrients associated with phytate were more difficult to clarify. The lack of effect of the enzyme on calcium digestibility was in contrast to published results, although the reasons for this discrepancy were unclear. Further work is needed in this area. In particular, the relationship between calcium level and phytate hydrolysis deserves attention, as recent studies (Mroz *et al* 1994) have demonstrated reduced hydrolysis of phytates by *Aspergillus niger* phytase in the presence of high calcium to phosphorus ratios.

It was expected that phytase would influence the digestibility of protein, since phytic acid forms insoluble protein-phytate - mineral complexes during processing and digestion (Knuckles *et al*, 1985), and also inhibits protease enzymes such as trypsin, tyrosinase and pepsin (Nair *et al*, 1991; Caldwell, 1992). However, effects of phytase on protein digestibility were inconsistent between trials; an increased protein digestibility was achieved with the barley-based diet used in the first experiment, but not with the corn-based diets of the subsequent trials. These discrepancies were similar to those found in the literature, and may have been due to the variable solubility of the phytate-protein complex, depending on the origin of the phytate.

Phytic acid is a precursor of many of the fibre components and possesses the antinutritional properties associated with fibre, such as formation of gels and reduced mineral absorption in the gut. The small intestine of pigs lacks the enzymes needed to cleave the bonds of non-starch polysaccharides. It was speculated that degradation of phytate should prevent some of the gel formation and mineral absorption associated with high-phytate diets. However, little effect of phytase on digestible energy content of the diet was observed, suggesting, in agreement with the literature, a lack of effect of the enzyme on fibre degradation. While it has been proposed that considerable fermentation may take place anterior to the hind-gut, suggesting that there may be an opportunity to increase fermentation and subsequent release of phosphorus, addition of yeast to the diet of growing pigs did not seem to be effective in achieving this.

Total hydrolysis of phytate was not observed, even when phytase was included at the level required for maximum response. This may have been due to incomplete hydrolysis of phytate intermediates. As indicated, the development of "potent" phytases which completely degrade intermediates, may be a challenge for the future. Addition of 1000 units of phytase to diets containing high levels of phytate-phosphorus allowed between 50 and 70% of the phytate-phosphorus to be utilised. Depending on the diet, this was equivalent to between 0.4 and 1.5g inorganic phosphorus (approximately). The equivalence was reduced at higher levels of inorganic phosphate, a factor which is not considered in commercial use of the enzyme. Current recommendations, which are based on titration results of 400 phytase units equivalent to 1g of inorganic phosphorus (Gropp, 1995), are an oversimplification, and should be re-addressed in light of the present results.

8.3 Reduction of phosphorus excretion

The study focused on reducing the phosphorus output of growing pigs, as they are responsible for a large proportion of total phosphorus output, but the enzyme may also be appropriate for finishing pig and sows diets. Reductions of 37% in faecal phosphorus output were found, in agreement with the literature and it is feasible that the total phosphorus output of the pig sector could be reduced by this much.

Legislation against phosphorus pollution is currently being adopted in many countries. The Netherlands was the first to introduce obligatory manure book-keeping, and fines for surplus output of phosphorus. In many other European countries, farmers must operate within a legal limit for a set number of animals per unit of land, and within the next decade, legislation of the European Union will be imposed. Phosphorus pollution is not only a European problem, but exists in many countries with a high population density of livestock. In Korea, for example, the problem has been recognised, and run-off from pig units must now be decreased by 40%. The net result of this legislation is a reduction of the number of animals that may be kept in an area, and while the cost of phytase is still a limiting factor, under these circumstances, use of the enzyme becomes a viable alternative to inorganic phosphates. Consensus over quantification of the increased digestibility of other nutrients associated with phytates may further increase the value of phytase.

Figure 8.1 Increase of net phosphorus requirement in response to daily liveweight gain (Whipp, 1994)

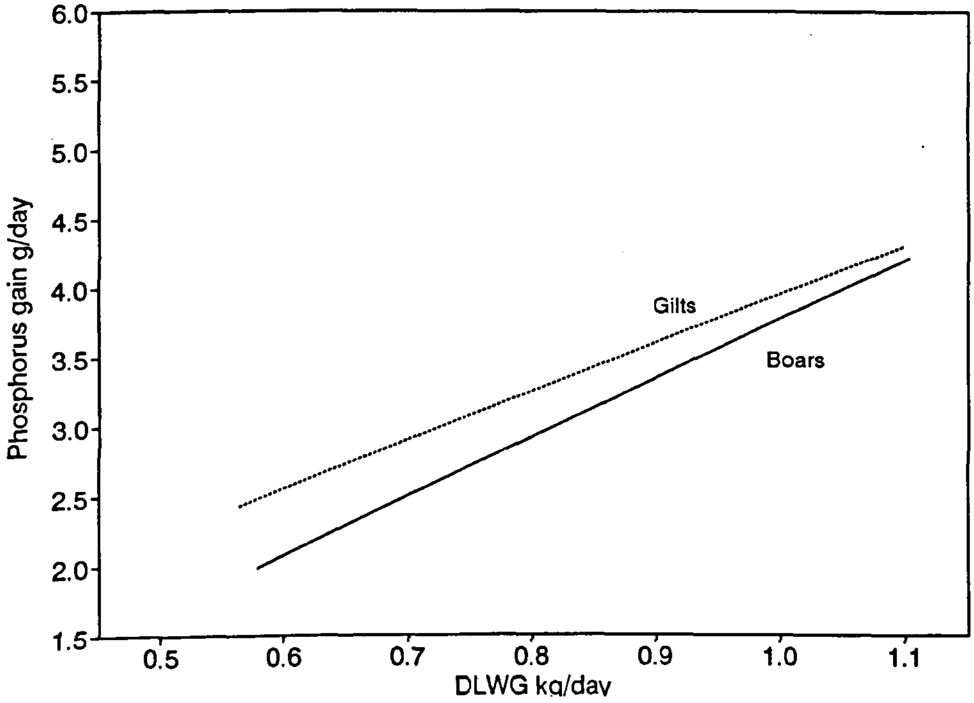

Table 8.1 Daily phosphorus requirements for growing pigs - comparison between results of present trials and published data

	ARC (1981)		NRC (1988	Gueguen and Perez, 1981			Jongbloed *et al*, 1993		Results of present trials	
Liveweight	25	45	20-50	35	50	70	30	50	10-25	25-60
Daily gain (kg)	0.55	0.79	0.70	0.60	0.75	0.80	0.57	0.82	0.63	0.89
P required g/day	4.6	5.2	9.5[r]	9.0[r]	11.0[r]	12.0[r]	3.12	3.44	5.50	9.0

[r] Total phosphorus required, assuming 50% availability

8.4 Conclusions

While the forgoing discussion included inferences from published literature, the following conclusions were drawn only on the basis of experimental work undertaken during the course of this project:

- Addition of *Aspergillus niger* phytase resulted in hydrolysis of phytates and increased availability of phytate-phosphorus in the digestive tract.
- A level of 1000 units/kg accomplished the maximum increase in phosphorus digestibility, although the economic optimum was 400 - 500 units/kg.
- Pigs growing from 10-25kg liveweight required 5.5g/day of digestible phosphorus, while 4g/day was sufficient for maximum bone strength.
- At least 9g digestible phosphorus/day was needed for maximum growth of pigs growing from 25-60kg. 6g/day was sufficient for maximizing bone strength.
- Phytase overcame the growth constraints of a phosphorus- deficient diet.
- Use of phytase enabled 50 to 70% of phytate-phosphorus to be utilised.
- The response of pigs to phytase was diet - dependent and influenced by the inorganic phosphorus level of the diet.
- Use of phytase in pig feeds could enable a reduction of one third in the phosphorus output from the growing pig sector.

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Appendix I Procedure during metabolism trials

Experiment 1 Cannulation trial

day		
1	Pigs weighed and feeding allowances calculated on basis of liveweight	
8	Pigs transferred to metabolism crates	
11	Dye added to evening meal	collection period I
12		
13		
14		
15		
16	Dye added to evening meal	
18	Ileal samples taken	
20	Ileal samples taken	
22	Ileal samples taken	
23	Pigs removed from crates, weighed, and feed allowance adjusted	
30	Pigs transferred to metabolism crates	
33	Dye added to evening meal	collection period II
34		
35		
36		
37		
38	Dye added to evening meal	
40	Ileal samples taken	
42	Ileal samples taken	
44	Ileal samples taken	
45	Pigs removed from crates, weighed and feeding allowances adjusted	
52	Pigs transferred to metabolism crates	
55	Dye added to evening meal	collection period III
56		
57		
58		
59		
60	Dye added to evening meal	
62	Ileal samples taken	
64	Ileal samples taken	
66	Ileal samples taken	
67	Pigs removed from crates, weighed, and feed allowance adjusted	
74	Pigs transferred to metabolism crates	
77	Dye added to evening meal	collection period IV
78		
79		
80		
81		
82	Dye added to evening meal	
84	Ileal samples taken	
86	Ileal samples taken	
88	Ileal samples taken	
89	Pigs removed from crates and trial ended	

Appendix I contd. Procedure during metabolism trials

Experiment 2 Dose-response metabolism trial

Day		
1	Pigs weighed and feeding allowances calculated on basis of liveweight	
8	Pigs transferred to metabolism crates	
11	Dye added to evening meal	collection period I
12		
13		
14		
15		
16	Dye added to evening meal	
18	Pigs removed from crates, weighed, and feed allowance adjusted	
25	Pigs transferred to metabolism crates	
28	Dye added to evening meal	collection period II
29		
30		
31		
32		
33	Dye added to evening meal	
34	Pigs removed from crates, weighed, and feed allowance adjusted	
41	Pigs transferred to metabolism crates	
44	Dye added to evening meal	collection period III
45		
46		
47		
48		
49	Dye added to evening meal	
50	Pigs removed from crates and trial ended	

Appendix II Trial 1 Veterinary records

Date	*Pig no.*	*Treatment*
25/5/92 1/5/92	1-6	1 ml 48% Tribrissen, pre and post-operative umbrella cover
27/4/92	1-6	General Anaesthetic prior to surgery
27/4/92	1-6	0.7ml Finadyne analgesic, post operative
29/4/92	1-6	" "
11/5/92	2,3,5,6	Local anaesthetic prior to draining abscess
12/5/92	2-6	Duplocillin penicillin for abscesses
13/5/92	1	3.5 ml Intramycetin
4/6/92	5	3.5 ml Intramycetin
5/6/92	5	3.5 ml Intramycetin

Animal losses

Pig no. 2

Sent to slaughterhouse on 2/7/92 as the cannula had come out overnight. No sign of discomfort or distress to the animal which was eating and drinking as normal

Pig no. 5

From 25/5/92 the pig started to refuse feed and showed general signs of ill health. At this stage, cannula leakage was not problematic, body temperature was normal and as there were no detectable signs of infection, pharmaceutics were not administered. By 4./6/92 there was no improvement and cannula leakage had increased. Intramycetin was administered, and a single injection of vitamin B12 was given to stimulate the liver. The cannula was flushed and ileal contents showed traces of blood. Intramycetin treatment was repeated the following day. After 24 hours there was no improvement and as the animal was by now very weak, a lethal injection of pentobarbitone was administered.

Appendix III Growth data of animals in Experiment 3

Pig no.	1	2	3	4	5	6	7	8
diet	1	2	3	4	5	6	7	8
Sex	G	B	G	B	G	B	G	B
Start date	06/02/93	06/03/93	06/03/93	06/12/93	06/02/93	05/31/93	06/05/93	06/07/93
Start weight	10	10.6	10.2	10	11.2	10.2	10.2	10.4
Finish date	07/05/93	06/28/93	07/05/93	07/12/93	06/28/93	06/28/93	07/05/93	07/05/93
Finish weight	26.2	25.8	28	28	26.2	26.2	26.4	30
Weight gain	16.2	15.2	17.8	18	15	16	16.2	19.6
Days on trial	33	25	32	30	26	28	30	28
DLWG	0.491	0.608	0.556	0.600	0.577	0.571	0.540	0.700
weights:								
06/07/93	11.8	12.9	12	7.9	13.2	12.4	11.6	10.4
06/14/93	14.9	16.7	15.8	11.1	16.8	16	14.4	15.1
06/21/93	18.4	21.2	19.6	14.8	21.6	21	18.6	20
06/28/93	22	25.8	24	19.2	26.2	26.2	22.2	24.5
07/05/93	26.2	-	28	23.6	-	-	26.4	30
07/12/93	-	28	-	-	-	-	-	-
Feed intake	26.15	22.14	30.00	28.26	23.30	20.70	26.10	28.33
Feed/day	0.79	0.89	0.94	0.94	0.90	0.74	0.87	1.01
FCE	1.61	1.46	1.69	1.57	1.55	1.29	1.61	1.45
Slaughter weight	25.60	24.20	26.40	26.40	25.00	24.60	24.80	28.40
Blood weight	1.28	1.18	1.28	1.32	1.22	1.16	1.18	1.36
Full gut weight	2.04	2.20	1.92	2.16	1.80	2.00	2.16	2.62
Empty gut weight	1.70	1.76	1.56	1.70	1.58	1.60	1.68	1.94
Carcase weight	21.06	19.92	22.52	23.02	21.04	20.84	20.96	23.64
Total carcase	24.38	23.30	25.72	26.50	24.06	24.00	24.30	27.62

Appendix III contd.

Pig no.	9	10	11	12	13	14	15	16
Diet	16	15	14	13	12	11	10	9
Sex	B	G	B	G	B	G	B	G
Start date	06/10/93	06/10/93	06/07/93	06/03/93	06/10/93	06/03/93	05/29/93	06/02/93
Start weight	10.1	10	10	10.2	10	10.5	10	10.8
Finish date	07/05/93	07/05/93	07/05/93	06/28/93	07/05/93	07/05/93	06/28/93	06/28/93
Finish weight	28.6	25	27.8	25.8	26.8	29	25.6	29.8
Weight gain	18.5	15	17.8	15.6	16.8	18.5	15.6	19
Days on trial	25	25	28	25	25	32	30	26
DLWG	0.740	0.600	0.636	0.624	0.672	0.578	0.520	0.731
weights:								
06/07/93	9.4	9	10	12.6	9.2	12.8	12.4	13.4
06/14/93	13.5	11.2	13.4	16.8	13.3	14.5	17	18.3
06/21/93	18.2	15.8	18	20.8	17.6	19.4	21	23
06/28/93	23.5	19	22.1	25.8	22.4	24.1	25.6	29.8
07/05/93	28.6	25	27.8	-	26.8	29	-	-
Feed intake	26.55	20.84	26.51	23.50	21.05	30.49	24.00	21.67
Feed/day	1.06	0.83	0.95	0.94	0.84	0.95	0.80	0.83
FCE	1.44	1.39	1.49	1.51	1.25	1.65	1.54	1.14
Slaughter weight	27.20	23.80	26.20	24.60	25.20	28.00	24.40	28.30
Blood weight	1.48	1.10	1.38	0.86	1.18	1.14	1.20	1.50
Full gut	2.26	2.00	2.30	2.22	2.58	2.32	1.78	2.48
Empty gut	1.74	1.44	1.80	1.70	1.76	1.74	1.52	1.92
Carcase weight	22.80	19.96	21.46	20.80	20.72	23.74	19.90	22.46
Total carcase	26.54	23.06	25.14	23.88	24.48	27.20	22.88	26.44

Appendix III contd.

Pig no.	17	18	19	20	21	22	23	24
Diet	6	5	4	3	2	1	1	17
Sex	G	B	G	B	G	B	G	B
Start date	06/02/93	06/07/93	06/05/93	05/31/93	05/31/93	06/07/93	06/02/93	06/03/93
Start weight	11.1	11.4	10	10.2	10	11	11	10
Finish date	06/28/93	06/28/93	07/05/93	07/05/93	06/28/93	07/05/93	06/28/93	07/05/93
Finish weight	27.2	25	28	29.6	25	26.4	26.4	29
Weight gain	16.1	13.6	18	19.4	15	15.4	15.4	19
Days on trial	26	21	30	35	28	28	26	32
DLWG	0.619	0.648	0.600	0.554	0.536	0.550	0.592	0.594
weights:								
06/07/93	13.9	11.4	10.3	9.6	13.4	11	13.6	11.9
06/14/93	16	15.8	14	13.2	16.6	14.4	17.4	16
06/21/93	22	20.4	18.2	18	20	17.8	21	20.2
06/28/93	27.2	25	23	24.3	25	22.6	26.4	24.6
07/05/93	-	-	28	29.6	-	26.4	-	29
Feed intake	23.46	19.94	27.09	27.96	22.36	23.05	22.85	28.55
Feed/day	0.90	0.95	0.90	0.80	0.80	0.82	0.88	0.89
FCE	1.46	1.47	1.51	1.44	1.49	1.50	1.48	1.50
Slaughter weight	26.00	23.80	26.00	28.00	23.00	25.40	24.50	28.00
Blood weight	1.16	1.32	1.44	1.42	1.14	1.32	1.18	1.44
Full gut weight	2.38	2.26	2.42	2.46	2.50	2.62	2.10	2.46
Empty gut	1.84	1.78	1.94	2.04	1.78	1.78	1.78	1.94
Carcase weight	21.50	19.40	20.90	23.54	18.24	20.40	20.52	23.54
Total carcase	25.04	22.98	24.76	27.42	21.88	24.34	23.80	27.44

Appendix III contd.

Pig no.	25	26	27	28	29	30	31	32
Diet	7	8	9	10	11	12	13	14
Sex	B	G	B	G	B	G	B	G
Start date	06/02/93	05/29/93	06/10/93	06/03/93	05/31/93	06/05/93	06/03/93	05/31/93
Start weight	11.4	10	10.1	10.8	10	10.3	10.2	10
Finish date	06/28/93	06/28/93	07/12/93	06/28/93	06/28/93	06/28/93	06/28/93	06/28/93
Finish weight	28.6	29.8	29.1	25.6	25.4	25.2	25.4	27.2
Weight gain	17.2	19.8	19	14.8	15.4	14.9	15.2	17.2
Days on trial	26	30	32	25	28	23	25	28
DLWG	0.662	0.660	0.594	0.592	0.550	0.648	0.608	0.614
weights:								
06/07/93	14.5	14.4	8.8	13	12.4	12.6	11.8	13.9
06/14/93	18.4	18.8	11.8	16.9	16.3	15.6	14.8	18.2
06/21/93	23.1	24.2	15.4	21.1	20.4	20.2	20	22.5
06/28/93	28.6	29.8	20.8	25.6	25.4	25.2	25.4	27.2
07/05/93	-	-	24.6	-	-	-	-	-
07/12/93	-	-	-	29.1	-	-	-	-
Feed intake	24.55	29.36	28.65	22.05	21.40	21.70	21.44	24.94
Feed/day	0.94	0.98	0.90	0.88	0.76	0.94	0.86	0.89
FCE	1.43	1.48	1.51	1.49	1.39	1.46	1.41	1.45
Slaughter weight	26.60	28.20	27.60	24.40	24.00	24.20	24.30	26.00
Blood weight	1.56	1.20	1.30	0.96	1.30	1.18	1.28	1.16
Full gut weight	2.86	2.66	2.88	2.68	2.12	2.32	2.22	2.28
Empty gut weight	2.04	2.00	1.98	1.80	1.66	1.98	1.74	1.76
Carcase weight	21.32	23.32	23.84	20.06	20.12	19.72	20.00	21.40
Total Carcase	25.74	27.18	28.02	23.70	23.54	23.22	23.50	24.84

Appendix III contd.

Pig no.	33	34	35	36	37	38	39	40
Diet	4	3	2	1	18	17	16	15
Sex	G	B	G	B	G	B	G	B
Start date	06/12/93	06/03/93	06/10/93	06/03/93	06/03/93	06/03/93	06/03/93	06/03/93
Start weight	10	10.8	11	10.8	10.2	11	10	12.8
Finish date	07/12/93	07/05/93	07/05/93	07/05/93	07/05/93	06/28/93	07/05/93	06/28/93
Finish weight	29	29.7	28	26.2	28.5	28	28.3	27.8
Weight gain	19	18.9	17	15.4	18.3	17	18.3	15
Days on trial	30	32	25	32	32	25	32	25
DLWG	0.633	0.591	0.680	0.481	0.572	0.680	0.572	0.600
weights:								
06/07/93	7.8	13	9.6	12.6	12	13.2	12.1	14.5
06/14/93	11	17	13.4	15.2	15.3	18	15.4	19.6
06/21/93	15	21	17.6	18.9	19.2	22.5	19.2	23
06/28/93	19.2	23.4	22.5	22.8	24.4	28	24.4	27.8
07/05/93	24.6	29.7	28	26.2	28.5	-	28.3	-
07/12/93	29	-	-	-	-	-	-	-
Feed intake	29.40	30.64	24.90	24.10	28.59	22.85	31.09	24.17
Feed/day	0.98	0.96	1.00	0.75	0.89	0.91	0.97	0.97
FCE	1.55	1.62	1.46	1.56	1.56	1.34	1.70	1.61
Slaughter weight	28.10	28.80	26.50	25.50	27.00	26.40	27.20	27.20
Blood weight	1.24	1.64	1.40	1.14	1.16	1.32	1.20	1.36
Full gut	2.18	2.46	2.58	2.18	2.00	2.20	2.30	2.32
Empty gut	1.64	2.02	1.74	1.58	1.60	1.68	1.84	1.82
Carcase weight	24.42	23.54	23.38	21.92	21.64	22.64	23.18	22.70
Total carcase	27.84	27.64	27.36	25.24	24.80	26.16	26.68	26.38

Appendix III contd.

Pig no.	41	42	43	44	45	46	47	48
Diet								
Sex	B	G	B	G	B	G	B	G
Start date	05/31/93	06/10/93	06/03/93	06/14/93	06/03/93	06/12/93	06/03/93	06/10/93
Start weight	10.8	11	11.5	11.2	10.9	11.2	11	10.6
Finish date	06/28/93	07/05/93	06/28/93	07/12/93	06/28/93	07/12/93	06/28/93	07/05/93
Finish weight	27.8	28.2	26.2	28	25	29.2	28.4	26.2
Weight gain	17	17.2	14.7	16.8	14.1	18	17.4	15.6
Days on trial	28	25	25	28	25	30	25	25
DLWG	0.607	0.688	0.588	0.600	0.564	0.600	0.696	0.624
weights:								
06/07/93	15.2	9.8	13.5	8.2	13.8	8.8	13.4	9.4
06/14/93	18.2	13.8	17	11.2	17.2	11.7	18.2	12.8
06/21/93	23	18	21.8	14.8	20.8	13.3	22.5	16.8
06/28/93	27.8	23.2	26.2	19.4	25	19	28.4	21.6
07/05/93	-	28.2	-	24	-	23.7	-	26.2
07/12/93	-	-	-	28	-	29.2	-	-
Feed intake	23.74	25.36	22.77	26.60	20.15	28.16	23.36	21.70
Feed/day	0.85	1.01	0.91	0.95	0.81	0.94	0.93	0.87
FCE	1.40	1.41	1.55	1.58	1.43	1.56	1.34	1.39
Slaughter weight	25.80	26.20	25.40	27.00	24.20	28.40	27.40	24.60
Blood weight	1.50	1.28	1.10	1.42	1.28	1.36	1.34	1.48
Full gut	2.82	2.32	2.52	2.30	2.00	2.30	2.46	2.46
Empty gut	1.90	1.78	1.72	1.80	1.60	1.76	1.92	1.92
Carcase weight	21.74	22.24	20.72	22.96	19.90	23.96	23.06	20.32
Total carcase	26.06	25.84	24.34	26.68	23.18	27.62	26.86	24.26

Appendix III contd.

Pig no.	49	50	51	52	53	54	55	56
Diet	13	14	15	16	17	18	1	2
Sex	G	B	G	B	G	B	G	B
Start date	06/16/93	06/07/93	06/12/93	06/07/93	06/07/93	06/03/93	06/14/93	05/29/93
Start weight	10.8	11.1	10.8	11.4	11.1	11.8	10.8	10.6
Finish date	07/12/93	07/05/93	07/05/93	07/19/93	07/05/93	06/28/93	07/12/93	06/21/93
Finish weight	25.8	28.2	26	27.8	26.8	27.8	25	25
Weight gain	15	17.1	15.2	16.4	15.7	16	14.2	14.4
Days on trial	26	28	23	43	42	28	25	23
DLWG	0.577	0.611	0.661	0.390	0.561	0.640	0.507	0.626
weights:								
06/07/93	1.1	8.3	11.4	11.1	14	8.2	15	
06/14/93	9.4	14.6	11.6	13.7	13.3	18.4	10.8	20.1
06/21/93	12.2	19	16	16	17.5	22.8	13.6	25
06/28/93	16.4	24	21	19	22.6	27.8	17	-
07/05/93	21	28.2	26	23	26.8	-	20.9	-
07/12/93	25.8	-	24.7	-	25	-		-
07/19/93	-	-	27.8	-	-	-	-	
Feed intake	23.10	27.00	21.85	28.93	23.20	22.12	21.45	18.63
Feed/day	0.89	0.96	0.95	0.69	0.83	0.88	0.77	0.81
FCE	1.54	1.58	1.44	1.76	1.48	1.38	1.51	1.29
Slaughter weight	24.60	27.20	24.00	27.20	25.30	26.60	23.60	24.00
Blood weight	1.92	1.54	1.22	1.24	1.26	1.14	1.22	1.20
Full gut weight	2.16	2.56	2.20	2.60	2.40	2.16	2.18	2.08
Empty gut weight	1.58	1.88	1.72	1.80	1.78	1.70	1.60	1.64
Carcase weight	21.12	22.84	20.28	22.50	21.16	22.80	20.22	19.92
Total carcase	25.20	26.94	23.70	26.34	24.82	26.10	23.62	23.20

Appendix III contd.

Pig no.	57	58	59	60	61	62	63	64
Diet	18	17	15	16	13	14	12	11
Sex	B	G	B	G	B	G	B	G
Start date	06/03/93	06/21/93	06/03/93	06/21/93	06/03/93	06/21/93	06/03/93	06/14/93
Start weight	10.2	10	11.2	10.6	11.1	11.8	10.110	
Finish date	06/28/93	07/19/93	07/05/93	07/19/93	06/28/93	07/12/93	06/28/93	07/12/93
Finish weight	26.6	28.6	26.8	28.2	27.4	27.2	26.2	28.8
Weight gain	16.4	18.6	15.6	17.6	16.3	15.4	16.1	18.8
Days on trial	25	28	32	28	25	21	25	28
DLWG	0.656	0.664	0.488	0.629	0.652	0.733	0.644	0.671
weights:								
06/07/93	13.4	-	13.9	-	14.2	-	13.2	-
06/14/93	17.6	7.4	16.7	8.1	17.3	9	17	10
06/21/93	22	10	20.6	10.6	22	11.8	21.2	12.6
06/28/93	26.6	14.6	24.6	15.2	27.4	16.5	26.2	17.5
07/05/93	-	18.8	26.8	19	-	21.4	-	22.8
07/12/93	-	23.6	-	23.6	-	27.2	-	28.8
07/19/93		28.6		28.2				
Feed intake	25.18	27.35	26.20	29.00	21.98	24.29	21.83	27.52
Feed/day	1.01	0.98	0.82	1.04	0.88	1.16	0.87	0.98
FCE	1.54	1.47	1.68	1.65	1.35	1.58	1.36	1.46
Slaughter weight	26.20	27.60	26.00	26.90	26.00	26.30	26.20	26.70
Blood weight	1.16	1.38	1.36	1.26	1.30	1.20	1.28	1.40
Full gut	2.28	2.62	2.02	2.48	2.54	2.58	2.28	2.58
Empty gut	1.58	1.88	1.64	2.02	1.70	1.98	1.68	2.02
Carcase weight	21.58	22.92	21.94	22.70	21.80	22.38	21.58	22.20
Total carcase	25.02	26.92	25.32	26.44	25.64	26.16	25.14	26.18

Appendix III contd.

Pig no.	65	66	67	68	69	70	71	72
diet	3	4	5	6	7	8	9	10
Sex	G	B	G	B	G	B	G	B
Start date	06/16/93	05/29/93	06/25/93	05/31/93	06/21/93	05/31/93	06/16/93	06/03/93
Startweight	10	10.8	10.8	10.6	10.8	10.8	10.9	11.4
FinishDate	07/12/93	06/21/93	07/26/93	06/28/93	07/19/93	06/28/93	07/12/93	06/28/93
Finish weight	26.5	25	28.4	26.4	28	26.4	25.4	29
Weight gain	16.5	14.2	17.6	15.8	17.2	15.6	14.5	17.6
Daysontrial	26	23	31	28	28	28	26	25
DLWG	0.635	0.617	0.568	0.564	0.614	0.557	0.558	0.704
weights:								
06/07/93	-	15.5	-	13.6	-	14.4	-	13.7
06/14/93	9.5	19.6	8	16.8	8	17.7	9.4	18.2
06/21/93	11.8	25	9.4	21.1	10.8	22	13.2	23.6
06/28/93	16	-	11.8	26.4	14.4	26.4	15.8	29
07/05/93	20.4	-	15.2	-	19.2	-	20.2	-
07/12/93	26.5	-	18.6	-	23.2	-	25.4	
07/19/93			23.2		28			
Feed intake	19.72	18.15	31.04	21.50	26.83	19.81	23.10	24.87
Feed/day	0.76	0.79	1.00	0.77	0.96	0.71	0.89	0.99
FCE	1.20	1.28	1.76	1.36	1.56	1.27	1.59	1.41
Slaughter weight	25.40	24.20	27.20	25.20	26.40	25.60	25.40	27.50
Blood weight	1.42	0.94	1.20	1.34	1.08	1.08	1.26	1.64
Full gut weight	2.36	2.28	2.20	2.38	2.28	2.46	2.64	2.62
Empty gut weight	1.90	1.64	1.62	1.98	1.86	1.88	2.12	1.96
Carcase weight	20.66	19.82	23.60	20.46	22.32	21.12	19.24	22.72
Total carcase	24.44	23.04	27.00	24.18	25.68	24.66	23.14	26.98

Appendix IV Trial 4 Veterinary Records

Scouring problems: the following were treated with 5ml Depocillin for up to 5 day or until scouring ceased

date	*Pig*
29/2/94	45 58 60
30/2/94	45 58 60 65
31/2/94	45 58 60 65
01/3/94	47 52 65
02/3/94	47 52 65
03/3/94	47 52 65
04/3/94	47

Pigs removed from trial:

Pig no.	Date removed	Reason for removal
29	24/3/94	Developed severe lameness with additional scouring problems. Treated for five days (20/3/94-24/3/94) with 5ml Lincocin; no improvement.
45	31/3/94	Scouring; lost condition at a rapid rate and was therefore removed from the trial.
48	19/3/94	Refused feed consistently although no signs of infection were evident. Showed aversion to diet.
57	27/4/94	Began to scour and refuse feed towards the end of the experiment.

Appendix V Growth data of animals in experiment 4

Pig no.	1	2	3	4	5	6	7	8
Diet	1	2	3	4	5	6	7	8
start weight	26	25	25.5	25	26.5	27	25	27.5
Start date	02/15/94	02/15/94	02/13/94	02/11/94	02/11/94	02/13/94	02/11/94	02/11/94
Finish weight	61.5	60	67.5	62.5	60	63	60	60
Finish date	04/19/94	04/05/94	04/05/94	04/05/94	03/29/94	03/29/94	03/22/94	03/15/94
Days	63	49	51	53	46	44	39	32
Feed intake	103.122	90.008	96.566	90.005	91.469	89.394	87.128	75.528
Daily FI	1.637	1.837	1.893	1.698	1.988	2.032	2.234	2.360
Gain	35.5	35	42	37.5	33.5	36	35	32.5
FCE	2.90	2.57	2.30	2.40	2.73	2.48	2.49	2.32
DLWG	0.559	0.730	0.793	0.732	0.688	0.812	0.877	1.012
Starved wt	59	58.5	66	61	59	62.5	58.5	58.5
Carcase wt	43.5	41.5	47.5	43.75	41.5	45.5	41.8	40.5
Carcase length	689	663	711	749	707	686	671	679
Max. shoulder	24.7	30.6	30	24.4	22.3	22.6	21	25.4
Min. midback	11.3	11	10.8	7.3	7	7.7	7.3	8.5
Ant. glut. med.	12.7	13	13.6	8.6	11.6	12.5	9	8.7
Min. glut. med.	3.7	9.1	7.1	6.1	6.1	4.8	3.1	7.1
Post. glut. med	7.9	14	12.4	8.4	7.8	10.1	9.2	12.7
P1	5.5	8	8.8	5.8	8.1	6.4	5	6.8
P2	5.5	7.3	8.3	6.5	7.1	6.5	4.8	5.6
P3	5.2	8.1	8.3	8	7.7	6.8	5	6.2
A	86.7	79	87.7	80.2	81	82.7	82.2	73.6
B	54.1	46.2	45.9	48.1	42.6	48.6	45.1	39.1
C	5.5	7.5	8.2	6.5	6.3	6.3	5	5.6

Appendix V contd

Pig no.	9	10	11	12	13	14	15	16
Diet	9	10	11	12	13	14	15	16
Start weight	25.5	25	26.5	25	27	27	31	26
Start date	02/15/94	02/11/94	02/15/94	02/13/94	02/15/94	03/01/94	03/08/94	02/15/94
Finish wt	65	64.5	61	62.5	64.5	63.5	64	71.5
Finish date	04/05/94	04/05/94	03/29/94	03/29/94	04/05/94	04/12/94	04/26/94	04/05/94
Days	49	53	42	44	49	42	49	49
Feed intake	110.742	115.311	83.747	83.6	103.618	96.202	106.048	121.041
Daily FI	2.260	2.176	1.994	1.900	2.115	2.291	2.164	2.470
Gain	39.5	39.5	34.5	37.5	37.5	36.5	33	45.5
Fce	2.80	2.92	2.43	2.23	2.76	2.64	3.21	2.66
DLWG	0.814	0.728	0.811	0.821	0.796	0.709	0.675	0.971
Starved wt	62.5	63	58	59.5	63.5	60.5	61	70.5
Carcase wt	41.75	44.6	41	43	46.25	44	45.5	51.35
Carcase length	696	700	697	683	699	681	688	725
Max. shoulder	23.2	28	24.8	26.2	26.6	23	20.5	25.3
Min. midback	7.2	9.1	6.4	9	12	8	7	10.6
Ant. glut. med.	11	8.9	7.3	12.6	12.3	8.4	10.9	9.1
Min. glut. med.	5.7	3.9	4.7	9.1	6.7	4.6	6	5
Post. glut. med	7.7	10.6	9.8	11.1	14.8	9	11.8	11.2
P1	9.9	6.1	7.2	6.2	6.5	6.3	4.6	7
P2	8.5	5.6	6.3	6.7	6.5	5.8	4.1	6.2
P3	8.5	5.6	5.3	8.6	9.4	6.6	4.1	6.6
A	79.1	86.1	81.6	80.6	80.7	80.8	89	83.6
B	46.4	47	39.8	45.4	46.6	46.8	53.1	51.6
C	8.5	5.7	5.1	6.7	6.1	5.8	5.2	6.4

Appendix V contd

Pig no.	17	18	19	20	21	22	23	24
Diet	17	18	10	9	8	7	6	5
Start weight	25	26	25	25	26	26.5	25	25
Start date	02/11/94	02/11/94	02/15/94	02/21/94	02/15/94	02/11/94	02/11/94	02/25/94
Finish weight	64.5	63.5	65.5	63.5	63	63.5	60	63.5
Finish date	03/29/94	03/29/94	04/19/94	05/18/94	04/12/94	03/29/94	03/29/94	04/12/94
Days	46	46	63	86	56	46	46	46
Feed intake	103.893	104.949	127.243	131.945	106.079	88.308	83.594	115.273
Daily FI	2.259	2.282	2.020	1.534	1.894	1.920	1.817	2.506
Gain	39.5	37.5	40.5	38.5	37	37	35	38.5
FCE	2.63	2.80	3.14	3.43	2.87	2.39	2.39	2.99
DLWG	0.877	0.831	0.631	0.476	0.708	0.805	0.761	0.848
Starved weight	62	61	63.5		61	61.5	58	62
Carcase weight	45.6	44.5	47	48	45	45.75	43	45.75
Carcase length	688	702	699	683	665	694	680	676
Max. shoulder	32.5	29.4	29.5	23.8	25	22.2	20.2	26.7
Min. midback	12.3	9.8	11	7.3	12.7	3.5	17.6	9.3
Ant. glut. med.	15.9	13	14.6	9	18.7	3.5	13	15.1
Min. glut. med.	13.4	8.7	2.4	3	11.4	2.3	5.2	10
Post. glut. med	17.9	16	9.7	8	14	7.8	6.8	16.7
P1	11.1	10.4	4.2	4.4	8.2	2.4	4.1	8
P2	10.8	9.3	4.2	3.6	6.9	3.1	3.9	8
P3	9.4	10.3	4.3	4.3	8	3.8	3.9	10.4
A	75.4	82.7	90.9	91	82.1	85.5	84.2	82.3
B	47	45	49.1	50.5	52	48.5	48.6	47
C	9.4	9.5	4.2	3.5	7.3	3.4	3.9	8

Appendix V contd

Pig no.	25	26	27	28	29	30	31	32
Diet	4	3	2	1	11	12	13	14
Start weight	26	26.5	26	26	-	25	27.5	25.5
Start date	02/15/94	02/15/94	02/13/94	02/15/94	-	02/15/94	03/01/94	02/15/94
Finish weight	60.5	62	61	61.5	-	66	61.5	61.5
Finish date	04/05/94	04/12/94	04/05/94	04/19/94	-	04/12/94	04/19/94	04/05/94
Days	49	56	51	63	-	56	49	49
Feed intake	95.27	104.277	104.306	115.685	-	122.278	101.68	101.77
Daily FI	1.944	1.862	2.045	1.836	-	2.184	2.075	2.077
Gain	34.5	35.5	35	35.5	-	41	34	36
FCE	2.76	2.94	2.98	3.26	-	2.98	2.99	2.83
DLWG	0.730	0.638	0.716	0.555	0.526	0.734	0.693	0.799
Starved weight	59.5	60	59.5	59	-	62.5	59.5	61.5
Carcase weight	42.85	46	41.75	43.5	-	46	45	44.6
Carcase length	682	690	701	682	-	689	678	702
Max. shoulder	29.4	23.2	24.6	25.1	-	28.6	26.1	29.9
Min. midback	9	7.4	5.6	7.6	-	10.6	13.3	9.7
Ant. glut. med.	13	6.7	6.4	13.7	-	12.8	11.3	12.3
Min. glut. med.	5.9	5.5	4	6.1	-	8.5	6.2	5.8
Post. glut. med	11.5	5.6	7.7	12.5	-	13.4	9.4	12.3
P1	7.9	6.5	6	6.8	-	8	5.4	8
P2	8.2	5.2	6	6.4	-	8	5.9	7.6
P3	8.2	5.3	6.4	6.8	-	7.8	8.2	8
A	86.7	86.3	79.5	82.2	-	78.8	87.2	80.2
B	51.2	50.7	45.3	52.8	-	48.4	50.5	43.2
C	7.2	5.3	6	6.4	-	8	5.9	7.6

Appendix V contd

Pig no.	33	34	35	36	37	38	39	40
Diet	15	16	17	18	11	12	13	14
Start weight	26	25	27.5	26	25	25.5	25	26.5
Start date	02/11/94	02/15/94	02/11/94	02/11/94	02/11/94	02/15/94	02/15/94	02/15/94
Finish weight	61.5	66.5	61.5	60.5	66	64.5	62.5	60.5
Finish date	03/29/94	04/12/94	03/29/94	03/29/94	04/12/94	04/12/94	04/26/94	04/05/94
Days	46	56	46	46	60	56	70	49
Feed intake	89.763	100.869	91.75	99.28	106.466	114.34	123.674	99.248
Daily FI	1.951	1.801	1.995	2.158	1.774	2.042	1.767	2.025
Gain	35.5	41.5	34	34.5	41	39	37.5	34
FCE	2.53	2.43	2.70	2.88	2.60	2.93	3.30	2.92
DLWG	0.764	0.759	0.783	0.814	0.628	0.737	0.556	0.739
Starved wt	59.5	63.5	58.5	58	61	63	61	59
Carcase wt	44	47	42.8	43	42.5	46	45.5	42.75
Carcase length	690	699	696	703	756	688	677	723
Max. shoulder	25.4	26.1	20.7	26	28.3	28.2	24.8	25.7
Min. midback	9.1	8	6.9	9.4	4.8	4.4	5.5	8
Ant. glut. med.	11.2	11.5	6	8.8	13.6	10.3	10.8	10
Min. glut. med.	5.7	4.8	3.5	5.7	6.4	6.4	6.5	3.7
Post. glut. med	9.4	10.6	7.7	9	14.3	11.3	11.9	6.7
P1	6	7.2	5	8.7	7.1	5.5	5.2	6.7
P2	6	6.4	5	7.5	7.1	5.7	4.2	6.7
P3	6.2	6.4	5.3	8.7	8.5	6.4	3.9	6.7
A	84.2	88.7	78.8	82.4	84.8	84.1	90.2	79.4
B	50.4	53	43.3	47	39.2	49	48.4	47
C	5.9	6.4	5.2	8.7	7.4	5.8	4.2	6.7

Appendix V contd

Pig no.	41	42	43	44	45	46	47	48
Diet	15	16	7	8	9	10	1	2
Start weight	25	27.5	28	25	-	25	27	-
Start date	02/13/94	02/15/94	02/11/94	02/11/94	-	02/15/94	02/25/94	-
Finish weight	61	60	63	67	-	60.5	60	-
Finish date	03/29/94	03/29/94	03/22/94	04/05/94	-	04/05/94	05/18/94	-
Days	44	42	39	53	-	49	82	-
Feed intake	90.155	78.63	91.411	103.109	-	93.778	131.135	-
Daily FI	2.049	1.872	2.344	1.945	0.000	1.914	1.599	0.000
Gain	36	32.5	35	42	-	35.5	33	-
FCE	2.50	2.42	2.61	2.45	-	2.64	3.97	-
DLWG	0.854	0.788	0.882	0.789	0.194	0.724	0.400	0.266
Starved weight	60.5	57	62	66	-	58.5	60	-
Carcase weight	44.5	42.8	43	47.5		42.5	45	
Carcase length	688	669	694	696		700	690	
Max. shoulder	28.4	23.4	27	26.5		28.3	22	
Min. midback	10.6	6.6	9.9	8.4		7.6	6.5	
Ant. glut. med.	15.3	6.5	11.3	7.5		8.9	7.1	
Min. glut. med.	10.1	3.4	7.2	5.6		3.5	2.5	
Post. glut. med	13.4	10.1	10.1	10.5		7.8	9.7	
P1	8.6	5.3	5.8	7.2		5.5	4.2	
P2	7	4.6	5.8	6.3		4.3	4.4	
P3	9.3	4.6	5.8	5.8		5	4.4	
A	72.5	82.1	76.8	92.1		82.3	88.1	
B	50	55.4	50.2	50		45.8	45.3	
C	6.4	4.6	5.8	6.3		4.3	4.4	

Appendix V contd

Pig no.	49	50	51	52	53	54	55	56
Diet	3	4	5	6	7	8	9	10
Start weight	25	26	25.5	25.5	27	25	26	27
Start date	02/15/94	02/11/94	02/11/94	02/13/94	02/11/94	02/15/94	02/11/94	02/25/94
Finish weight	60	63	61	63	62	60	63.5	62.5
Finish date	04/05/94	03/22/94	04/05/94	04/12/94	03/29/94	04/05/94	04/05/94	04/26/94
Days	49	39	53	58	46	49	53	60
Feed intake	87.263	95.692	90.727	105.509	100	77.427	103.9	116.354
Daily FI	1.781	2.454	1.712	1.819	2.174	1.580	1.960	1.939
Gain	35	37	35.5	37.5	35	35	37.5	35.5
FCE	2.49	2.59	2.56	2.81	2.86	2.21	2.77	3.28
DLWG	0.732	0.972	0.695	0.649	0.786	0.733	0.756	0.594
Starved weight	56.5	61.5	60.5	60.5	59.5	57.5	61	60.5
Carcase weight	40.2	44.75	44.7	43.75	44.5	38.8	44.35	44
Carcase length	688	681	663	721	686	693	706	686
Max. shoulder	25.5	29.3	23.9	27.1	27.3	23.8	27.6	22.8
Min. midback	8.7	11.3	8.2	8.9	10.2	7	10.8	5.8
Ant. glut. med.	13.3	18.1	9.1	10.9	13.4	4.2	10.8	12
Min. glut. med.	8.5	14.2	5	5.9	7.2	3.3	5.6	5.5
Post. glut. med.	11.6	16.6	10.9	9.2	18.9	5.1	9	11
P1	8.4	9.8	5.1	5.9	7.5	4.7	7.6	6.1
P2	7.1	8.5	3.8	7	7.3	3.9	7	6.5
P3	7.1	8.4	3.8	7.1	5.6	4.7	7	6.5
A	77.5	83.3	87.9	80.3	81.6	75.5	82.5	84
B	39.1	45.6	51.7	43.7	44.6	41.9	47.6	51.2
C	7.1	8.4	3.8	6.3	7	3.9	6.7	6.5

Appendix V contd

Pig no.	57	58	59	60	61	62	63	64
Diet	17	18	1	2	3	4	5	6
Start weight	-	26	26	27	27	27.5	27	27
Start date	-	03/01/94	02/19/94	02/19/94	02/25/94	03/01/94	03/01/94	02/19/94
Finish weight	-	60.5	60	62.5	63	62	61	65.5
Finish date	-	05/18/94	04/26/94	04/26/94	04/19/94	04/26/94	04/26/94	04/12/94
Days	-	78	66	66	53	56	56	52
Feed intake	-	129.497	110.927	104.269	99.127	88.887	101.485	99.887
Daily FI	-	1.660	1.681	1.580	1.870	1.587	1.812	1.921
Gain	-	34.5	34	35.5	36	34.5	34	38.5
FCE	-	3.75	3.26	2.94	2.75	2.58	2.98	2.59
DLWG	0.587	0.477	0.491	0.499	0.651	0.647	0.586	0.717
Starved weight	-		58	61	62	60.5	58.5	61.5
Carcase weight		44.75	41.5	44.25	45.75	43.75	43.5	45.75
Carcase length		708	715	692	687	750	694	700
Max. shoulder		21.2	32.6	25.9	24.1	26	28.3	24.7
Min. midback		7.6	7	13.2	8	8.6	8.3	8.1
Ant. glut. med.		4	15.3	13.7	12.9	10.3	14.9	11.7
Min. glut. med.		2	8.1	15.5	6.4	3.8	5.7	8.3
Post. glut. med		3.3	11.6	*	8.4	7.4	7.2	12.5
P1		4.2	6.5	7.3	6	3.8	5.4	7.2
P2		3.9	7	8.4	5.4	3.7	5.6	6.4
P3		4.2	6.4	11.2	5	3.3	5.9	7.8
A		82.2	89.9	83.6	89.1	85.3	85.9	83.8
B		49.9	40.5	49.4	50.4	47.6	44.9	45.8
C		3.9	7	8.7	5.4	3.7	5.6	6.4

Appendix V contd

Pig no.	65	66	67	68	69	70	71	72
Diet	15	16	17	18	11	12	13	14
Start weight	25.5	25.5	25	26	25.5	27	28	26.5
Start date	02/25/94	02/19/94	02/25/94	02/15/94	02/25/94	02/19/94	02/25/94	02/19/94
Finish weight	62	62.5	60	63.5	66.5	62	65	65.5
Finish date	04/26/94	04/12/94	04/19/94	04/19/94	04/26/94	04/12/94	04/12/94	04/12/94
Days	60	52	53	63	60	52	46	52
Feed intake	91.936	81.689	99.127	99.22	132.496	78.65	77.928	82.011
Daily FI	1.532	1.571	1.870	1.575	2.208	1.513	1.694	1.577
Gain	36.5	37	35	37.5	41	35	37	39
FCE	2.52	2.21	2.83	2.65	3.23	2.25	2.11	2.10
DLWG	0.534	0.740	0.632	0.610	0.623	0.682	0.823	0.785
Starved weight	59	59	57.5	60.5	64.5	57.5	61	61.5
Carcase weight	42.5	43	40.25	43.25	47.75	42.5	45	43.75
Carcase length	690	696	706	674	696	686	696	703
Max. shoulder	23.4	24.7	23.8	27.1	29.5	24.9	24.7	30.7
Min. midback	5.4	8.6	5.7	8.8	5.3	4.3	9.8	9.3
Ant. glut. med.	10.6	8.1	12.6	11.3	11.2	13.9	9.8	11.4
Min. glut. med.	5.2	4.5	8.9	9	6.6	6.5	5.6	5.4
Post. glut. med	12.1	10.2	13.9	13.4	14.6	10.4	11.1	13.7
P1	3.7	5.9	6.3	4.5	6.3	7.3	7.1	8.1
P2	3.2	6.1	5.7	3.8	5	5.5	6.1	8
P3	3.4	6.5	8.4	5	5.9	6.4	6.1	8.6
A	87.6	81.9	80.5	90.6	67.4	79	86	85.9
B	52.8	52.4	42	42.6	48.4	40.7	49.2	47.6
C	3.5	6.1	6.3	3.8	6	5.3	5.8	8