PHYTASE SUPPLEMENTATION ON THE AVAILABILITY OF DIFFERENT MINERALS AND THEIR INTERACTIONS IN PIGS

By

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Dedicated to my Family

DECLARATION

I hereby declare that the thesis entitled **"PHYTASE SUPPLEMENTATION ON THE AVAILABILITY OF DIFFERENT MINERALS AND THEIR INTERACTIONS IN PIGS"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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Certified that the thesis entitled **"PHYTASE SUPPLEMENTATION ON THE AVAILABILITY OF DIFFERENT MINERALS AND THEIR INTERACTIONS IN PIGS"** is a record of research work done independently by Dr. K. Shyama, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associationship to her.

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Introduction

1. INTRODUCTION

India ranks fifth in world meat production and the share of pigs in the total meat production was 10 per cent during the year 2003. The per capita availability of meat is only 5.5 kg/annum which is almost half of the ICMR recommendation of 10.8 kg meat /year. Pigs being highly prolific with very fast growth and high feed conversion efficiency can help to fill up the large gap between animal protein requirement and availability in India. Kerala is unique among the other states of the country in having a majority of non vegetarians, high literacy and without much religious taboos. Thus swine rearing is an enterprising livelihood of farmer sector in Kerala.

Minerals play a wide variety of roles structurally, electrochemically as well as catalytically inside the body. The concentration of minerals in crops and forage plants depends mainly on the species of plant, types of soil, season of crops and plant maturity. Cereal grains which form the major part of swine ration are generally low in calcium, while phosphorus is present in all common feed stuffs. Phytic acid (inositol hexaphosphoric acid) is an ester formed by combination of the six alcoholic groups of inositol with six molecules of hexaphosphoric acid and its salt phytate are the plant storage forms of phosphate. Phytates constitute over 60 per cent of the total phosphorus found in cereal grains and oil seeds and the availability of phosphorus from plant feeds is low compared to that from inorganic mineral supplements or supplements of animal origin. Ruminants are able to readily utilize phytate phosphorus due to the presence of microbial phytase. But in case of nonruminants, particularly the young ones phytate phosphorus is unavailable. Phytates also form insoluble complexes with Ca, Mg and other minerals affecting their availability in monogastric animals.

Dietary addition of phytase in pigs is documented to release a large portion of naturally occurring phytate phosphorus and thus greatly reduce the amount of inorganic phosphorus to be added in the pig diet. It is also used as a tool to reduce phosphorus excretion and thus reduce the environmental pollution. The dietary requirements of minerals are very difficult to be defined than for organic nutrients because many factors determine the utilization of minerals, such as interaction among minerals, the actual amount of mineral in the diet, the mineral status of the animal and form in which they occur.

In swine rearing, feed cost forms more than 75 per cent of cost of production. Under the field condition, pigs are reared on kitchen/hotel wastes alone without any mineral or vitamin supplementation. Studies on mineral requirement of the cross bred pigs prevailing to the present management conditions are scanty in literature. Hence a technical and scientific feeding regime to meet the nutritional requirements of the animals according to their management conditions can very well add to the efficiency of production. Under this background the present study was undertaken for the doctoral research programme with the following objectives.

To study

- The effect of different levels of calcium and phosphorus on growth
- The interactions of calcium and phosphorus on other minerals such as magnesium, manganese, zinc and copper
- The effect of phytase supplementation on the availability of different minerals.

Review of Literature

2. REVIEW OF LITERATURE

2.1 EFFECT OF DIFFERENT LEVELS OF MINERALS ON

2. 1. 1 Growth

Pond *et al.* (1978) observed that daily gain and feed intake of rats were higher with high Ca than with low Ca diets. They also reported that there was no effect of dietary Cu or Zn level on daily gain and feed intake. Kornegay *et al.* (1981a) found no overall difference in daily gain and daily feed intake between boars fed diets containing Ca and P at 100, 125 and 150 per cent of NRC recommendations and with two protein levels. They did not observe any interactions between the dietary Ca, P and protein content in the diet. Kornegay *et al.* (1981b) observed in another experiment in swine that mineral content of hair could be influenced by the dietary mineral levels but since numerous factors influenced the mineral content of hair, it is of limited value for the assessment of mineral status of animals. O'Quinn *et al.* (1997) observed that decreasing dietary phosphorus content by 25 per cent of NRC recommendations did not affect average daily gain (ADG) and feed intake during growth period in terminal cross growing-finishing pigs fed sorghum-soyabean meal based diets from 25 to 80 kg body weight.

Reinhart and Mahan (1986) studied the effect of various Ca and P ratios for starter, grower and finisher swine and reported that pigs fed low P diets displayed lower daily gains when the Ca:P ratio exceeded 1.3:1, whereas when high dietary P was provided, adverse effects on performance were not observed until the Ca:P ratio exceeded 2.0:1. The results suggested that high Ca:P ratios can adversely affect swine performance and bone development. Liu *et al.* (1998) also found that lowering the dietary Ca : total P (Ca:tP) ratio from 1.5:1 to 1.0:1, linearly increased ADG during the growing phase, but had no effect on growth performance during the finishing phase.

Nimmo *et al.* (1981) could not observe any effect on ADG by dietary treatments with 0.65 per cent Ca and 0.50 per cent P or 0.975 per cent Ca and 0.75 per cent P in gilts. They observed a lower daily feed intake for gilts fed low Ca:P diet. They concluded that 0.65 per cent Ca and 0.50 per cent P were not adequate to meet their requirements during growth from 7 to 93 kg body weight. They further opined that 13g Ca and 10g P/day during gestation were not adequate to meet Ca and P requirements of gilt during growth and gestation compared to 19.5g Ca and 15gP/day. Crenshaw (1986) observed that dietary Ca levels between 0.6 per cent and 1.2 per cent and dietary P levels between 0.6 per cent and 0.8 per cent did not adversely affect growth or bone development in growing swine. Cera and Mahan (1988) observed maximum gains at 0.65: 0.50 per cent and 0.52: 0.40 per cent Ca:P level during the grower and finisher periods, respectively.

Vipperman *et al.* (1974) suggested the requirements to be 0.75 per cent Ca and 0.50 per cent P in growing pigs as evident from their excretion and retention data. According to Hastad *et al.* (2004) grower (33 to 55kg) and finisher (88 to 109kg) pigs require 0.22 and 0.19 per cent available phosphorus (aP) respectively .

2.1.2 Feed Efficiency

Kornegay *et al.* (1981b) investigated the effects of three dietary Ca and P levels and two protein levels on feed lot performance of crossbred pigs and found no overall difference in feed per unit gain between boars fed diets containing 100, 125 and 150 per cent of NRC requirements of Ca and P. Nimmo *et al.* (1981) also could not observe any effect on feed /gain by two dietary treatments containing 0.65 per cent Ca and 0.5 per cent P or 0.975 per cent Ca and 0.75 per cent P in gilts. Crenshaw (1986) also opined that Ca levels between 0.6 per cent and 1.2 per cent and dietary P levels between 0.6 and 0.8 per cent did not adversely affect efficiency of gain. Reinhert and Mahan (1986) evaluated the effects of various Ca:P ratios at two dietary P levels (0.50 and 0.70, 0.40 and 0.60, 0.35 and 0.50) respectively for starter, grower and finisher diets) and found that pigs fed low P diets displayed lower feed efficiency after the Ca:P ratio exceeded 1.3:1, whereas negative effects of feeding high dietary P levels were exhibited only when the Ca:P ratio exceeded 2.0:1. Cera and Mahan (1988) evaluated the effects of feeding various dietary Ca:P levels (0.45:0.32, 0.52:0.40, 0.65:0.50 per cent) on feed efficiency in swine and they observed no difference between feed:gain ratios between the three dietary mineral treatments.

Mavromichalis *et al.* (1999) conducted three experiments to determine the effects of omitting vitamin and trace mineral premixes and or reducing inorganic P additions to finishing diets on growth performance in pigs found that removing vitamin and trace mineral premixes from diets during late finishing had no effect on gain:feed ratio.

Hastad *et al.* (2004) in a study to identify aP requirements of pigs reared in commercial facilities suggested that complete removal of supplemental P in diets for finishing pigs decreased gain:feed ratio.

2.1.3 Digestibility of Nutrients

Llyod *et al.* (1961) studied the effect of increasing levels of Ca (1.2, 2.0, 3.0 and 4.0 per cent) and addition of Oleandomycin on the performance of early-weaned pigs and observed that increasing Ca levels caused significant decrease in apparent digestibility of total carbohydrate while Oleandomycin brought about a significant increase in the apparent digestibility of gross energy (GE), crude protein (CP) and ether extract (EE). O'Quinn *et al.* (1997) observed in growing-finishing pigs weighing from 25 to 118 kg body weight fed sorghum-soyabean meal based diets that decreasing dietary P did not affect apparent digestibility of dry matter (DM) and nitrogen (N).

2.1.4 Mineral Availability

O'Quinn *et al.* (1997) conducted growth and digestion experiments to estimate the digestible P needs of growing-finishing pigs weighing 25 to 118 kg fed sorghum-soyabean meal based diets, results of which indicated an increase in apparent digestibility of P as dietary P decreased in the diet. They suggested that P excretions of terminal cross pigs can be reduced by feeding less than current NRC (1988) recommendations for pig without reducing performance.

2.1.5 Blood Parameters

Hoefer *et al.* (1960) studied the effect of adding Zn (50 or 75 ppm), Fe (100 ppm) and Cu (125 ppm) to control rations varying in Ca level (0.55, 1.05 and 1.317 per cent) in pigs and they observed increased serum alkaline phosphatase (ALP) activity due to Zn. Cromwell *et al.* (1970) reported that by increasing the dietary P from 0.34 to 0.56 per cent to pigs weighing 46 kg and from 0.29 to 0.44 per cent thereafter resulted in a linear increase in serum P and a linear decrease in serum Ca.

Kornegay *et al.* (1981b) in their study on influence of dietary Ca and P levels on serum minerals observed that serum P of barrows, boars and gilts were reduced when less than NRC suggested levels of P were fed. Kornegay *et al.* (1981a) observed that serum P was decreased as dietary Ca and P increased, with no difference in serum Ca and Mg, and there was no effect for protein levels. They also noted no interaction between dietary Ca and P and protein levels. Boyd *et al.* (1983) evaluated plasma ALP as a criterion for estimating aP in feedstuffs for swine. It was found that the plasma ALP increased with time and with the concentration of tP (0.26 to 0.50 per cent) indicating ALP as a sensitive index in P availability studies. Reinhart and Mahan (1986) studied the effect of various Ca:P ratios (1.3:1, 2.0:1, 3.0:1, 4.0:1) at low and high dietary P for starter, grower and finishing swine and found that serum Ca concentrations were higher and serum P concentrations were lower with increasing Ca:P ratio, with the response more pronounced at low dietary P levels.

Pond *et al.* (1966) in one of their experiment studied the effects of supplemental Zn, corn oil and cadmium on body weight gain, incidence of parakeratosis and levels of blood constituents and noted that serum ALP was greatly reduced after 4 and 6 weeks in pigs fed the low Zn diets, but there was no effect of added corn oil on this constituent despite the absence of parakeratosis. Hoekstra *et* *al.* (1967) fed corn-soyabean meal diet containing high Ca (1.6 per cent) with added Zn (100 ppm), and observed significantly higher activities of serum ALP than that of unsupplemented group.

Pond *et al.* (1978) reported that diets severely deficient in Ca can be tolerated by the finishing pig and growing rat without drastic effect on net uptake of tissue Ca, Cu, Fe, Mg, Mn and Zn even in association with wide variations in diet Cu and Zn levels.

2.1.6 Bone and carcass characteristics

Cromwell *et al.* (1970) observed that increasing dietary P from 0.34 to 0.56 per cent for pigs weighing upto 46 kg and from 0.29 to 0.44 per cent for pigs weighing above 46 kg improved bone ash values linearly. They also found that carcasses of pigs fed the low P diets tended to have smaller *Longissimus dorsi* areas than those of pigs fed at higher P levels. Carcasses of barrows fed the low P diet had significantly more back fat, smaller *l. dorsi* areas and lower yield of ham and loin than those of pigs fed the higher P levels. Pond *et al.* (1978) concluded in their study using growing Yorkshire pigs that pigs weighing more than 35 kg initially can adapt to dietary Ca and P levels and ratios considerably above or below NRC recommendations without any negative effects in concentrations of Ca, Mg, Cu, Mn and Zn in bone ash or in liver and kidney, although it is generally recognized that a Ca:P ratio of less than 1:2 or greater than 2:1results in abnormal bone.

Kornegay *et al.* (1981a) studied the influence of dietary Ca and P levels on bone development in barrows, gilts and boars and observed that serum P and bone mineralization were reduced when less than NRC suggested levels of P were fed and foot soundness scores were not influenced by growth rate or dietary Ca and P levels. They also noted that barrows, boars and gilts responded in a similar manner to higher than NRC suggested levels of Ca and P, and maximization of bone development occurred when pigs were fed at 125 per cent higher NRC levels of P and Ca.

Reinhart and Mahan (1986) in their study on effect of various Ca and P ratios (1.3:1, 2.0:1, 3.0:1, 4.0:1) at low and high dietary P (0.05 per cent below NRC, 1.0 per cent above NRC) for starter, grower and finishing swine found that per cent bone ash decreased as Ca:P ratio widened during the starter and grower production phases, with minimal response for finisher-phase bone ash. Dietary P level had a greater effect on per cent bone ash than Ca:P ratio, with high dietary P resulting in increased per cent bone ash in the starter, grower and finisher phases. Cera and Mahan (1988) also found that leg soundness at 56 kg and 95 kg body weight revealed no effect of dietary Ca:P level on soundness scores at 56 kg, but the interaction between grower and finisher diets was significant at 95kg body weight. Percentage bone ash of the humerus, shaft thickness and bending moment of the femur increased as dietary Ca:P level increased at both 56 kg and 95 kg body weights.

O'Quinn et al. (1997) opined that carcass yield and leanness in growing finishing pigs fed sorghum-soyabean meal based diets were unaffected by dietary P levels. Similarly Mavromichalis *et al.* (1999) reported that omitting the vitamin and trace mineral premixes had no effect on carcass backfat thickness and marbling, colour and firmness of the longissimus muscle in pigs. They also observed that diets containing Ca and P levels of 0.56 and 0.46, 0.51 and 0.40 and 0.47 and 0.31 per cent respectively, had no effect on meat quality or back fat thickness. Hastad *et al.* (2004) in a study to identify aP requirements of pigs reared on commercial facilities noted that pooled bending moment of the femur, sixth rib and third and fourth metatarsals increased with increasing aP. They also noted that increasing aP increased metacarpal bone ash percent and bending moment.

Kanakov *et al.* (2005) concluded in their study in pigs that bone development was significantly affected by dietary phosphorus level. They noticed that the low phosphorus diet gave significantly poor results than the adequate-phosphorus supplemented diet though there was no beneficial effect of supplementing P at a level higher than 2.4 g/kg and lowering the P level to 1.6 g/kg in the late finishing stage produced deleterious effects. Lyberg *et al.* (2006) in their study on effect of liquid feeding, P levels and phytase supplementation on digestibility and performance noted that low P diets resulted in lower femur density than high P diets. They further noted that soaking the diets for 1 h in room temperature (Water to feed ratio was 3:1in liquid diets) increased carcass weights in pigs fed a low phosphorus diet to the same level as pigs fed high phosphorus diets.

2.2 EFFECT OF PHYTASE SUPPLEMENTATION ON

2.2.1 Growth

Lei *et al.* (1993) conducted two experiments using corn-soyabean meal based diets containing 0 and 1350 units/g of supplemental microbial phytase and three levels of Zn in weanling pigs and the results indicated that supplemental phytase significantly enhanced weight gain and feed intake while supplemental Zn alone did not directly promote performance. Cromwell *et al.* (1993) fed pigs with fortified corn-soyabean meal diets containing adequate (0.5 per cent) and low (0.4 or 0.3 per cent) P during growing phase followed by 0.4 and 0.3 per cent P in the finishing phase and observed that though P deficient diets decreased growth rate in pigs, phytase supplementation (500 units/g) of the low P diets restored growth rate as that of control diet. Adeola *et al.* (1995) also observed that addition of phytase at 1500 (PU) / kg of diet, regardless of the level of Zn supplementation resulted in an increase in the rate of body weight gain in pigs. Qian *et al.* (1996) studied the effect of phytase supplementation to rations with wide Ca:P ratios in weanling pigs and found decreased average daily feed intake and ADG. They further reported that the activity of phytase was decreased as the Ca:tP ratio became wider. They also noticed that narrowing the dietary Ca:t P ratio from 2.0:1 to 1.2: 1 led to 16 per cent increase in phytase efficacy for improving performance. Biehl and Baker *et al.* (1996) on the other hand observed increased weight gain with phytase supplementation (1,200 PU/kg) to a vitamin D_3 adequate, P deficient corn-soyabean meal diet.

Harper and Kornegay (1996) observed better growth performance in pigs when graded levels of 167, 333 and 500 PU of phytase were supplemented per kg of low P diets. Murry *et al.* (1997) also found a linear increase in final body weight with increasing levels of microbial phytase (0, 700, or 1,000 PU/kg of diet) added to low P diets. Liu *et al.* (1997) also observed increased daily feed intake and daily gain in barrows with increasing levels of microbial phytase (0, 250 and 500 PU/kg of diet) added to a low P corn-soyabean meal diet. Similarly O'Quinn *et al.* (1997) studied the efficacy of a recombinant derived microbial phytase in sorghum-soyabean meal based diets of finishing swine and documented that supplemental P and phytase increased growth rate. Han *et al.* (1997) compared the effect of microbial phytase, cereal phytase or inorganic P supplementation to basal diets in pigs and found that the overall ADG was approximately 33 per cent greater than that of the control pigs fed only the basal diet and also the overall average daily feed intake was identical among pigs receiving the three dietary treatments. Harper *et al.* (1997) observed that supplementing the low P diet with either 250 or 500 PU/kg of Natuphos phytase resulted in substantial improvement in performance in growing-finishing crossbred pigs.

Matsui *et al.* (2000) observed that growing pigs given 1000 PU/kg of *Aspergillus niger* phytase showed greater ADG than those given yeast phytase in corn-soyabean meal diets. Sands *et al.* (2001) conducted a study in pigs with corn-soyabean meal based diets containing high available P (HAP) corn or normal corn with 600 units of phytase or without phytase in a 2x2 factorial experiment and observed higher body weight and rate of gain in pigs fed HAP corn phytase supplemented diet when compared to pigs fed normal corn. Landblom *et al.* (2002) observed a better growth performance among pigs receiving a reduced P diet plus allzyme phytase than that of control pigs. They could also observe improved ADG when the two enzymes allzyme and fibrozyme fed together. Similarly Gentile *et al.* (2003) evaluated a new biosynthetic, heat-stable enzyme derived from the sequences of multiple homologous phytases and found linear and quadratic responses in growth performance in pigs supplemented at 0 to 1,250 PU/kg of corn-soyabean meal diet without supplemental inorganic P. Similarly Omogbenigun *et al.* (2004) observed that pigs fed phytase supplemented diets elicited a higher ADG than those fed the control diet.

Shelton *et al.* (2004) used low Ca and low aP diet and found that adding phytase (500 PU/kg of diet) increased ADG when compared to that of unsupplemented groups. The ADG obtained was similar to those fed control diet with adequate Ca and aP, in pigs during all phases of growth. They also found that the average daily feed intake was not affected by level of Ca and aP with or without phytase. In another experiment Augspurger *et al.* (2004) observed in pigs that weight gain was increased linearly in response to graded levels of inorganic P (iP) supplementation (0, 0.075, 0.150 per cent) and phytase supplementation (500 and 1,000 PU/kg) while supplemental Zn (1,500 mg of Zn/kg) did not improve growth performance. Jendza *et al.* (2005) also found that addition of dietary phyatse (500 and 1000 PU/ kg) in basal diet linearly increased ADG in pigs without affecting average daily feed intake. Kies *et al.* (2005) also observed higher weight gains in piglets when fed diets supplemented with phytase at 1500 PU/kg feed than that of the control, without affecting feed intake. Brana *et al.* (2006) observed a linear increase in ADG in grower and finisher pigs subsequent to dietary addition of graded levels of Phyzyme (250, 500, 750, or 1,000 PU/kg) and Natuphos (250 500 and 1,000 PU/kg) and there was no difference for growth performance between the two enzymes (Phyzyme and Natuphos) at the same inclusion level. Subsequently Kies *et al.* (2006) observed an increase in average daily feed intake and ADG with increasing phytase levels from 100, 250, 500, 750, 1,500 to15,000 PU/kg feed.

Veum *et al.* (2006) observed that the addition of a genetically engineered *E.coli* phytase in increasing dietary concentrations from 0 to 12500 PU/kg to diets containing low aP (0.2 and 0.13 per cent) led to linear and quadratic increases in

ADG, for the entire experimental period. Guy *et al.* (2008) observed that feed intake and growth rate in weaner pigs were increased by phytase supplementation, either alone or in combination with organic acids (formic, fumaric and propionic)

Augspurger *et al.* (2003) found that supplementation of 500 PU / kg of ECP resulted in superior weight gain in chicks when added to corn-soyabean meal diets containing no supplemental iP when compared to those fed diets supplemented with Natuphos and Ronozyme. Results revealed P release values of 0.032 and 0.028 for 500 PU/kg Natuphos and Ronozyme respectively and 0.125 per cent for ECP.

Hariharan (2003) observed increased body weight in broiler chicks with addition of citric acid (3.0 per cent) or phytase (700 U/kg diet) or their combination (1.5 per cent citric acid + 350 U phytase/kg diet) to low aP diets (0.3 per cent). Subsequently Augspurger and Baker (2004b) observed that weight gain increased quadratically with increasing levels (0, 0.05, 0.10, 0.15, and 0.20 per cent) of supplemental iP in chicks. They also documented that weight gain was higher for chicks fed ECP than those fed fungal phytase1 (FP1) at 500 and 1000 PU /kg but were similar between the two phytases at higher phytase activity levels. In another trial in which the dietary Ca level was increased from 0.75 to 1 per cent they noticed that weight gain increased quadratically in response to supplemental iP along with ECP, FP1 and FP2 phytase supplementation. Dilger *et al.* (2004) observed that addition of 500 or 1,000 PU/kg of diet of a new microbial phytase (Phyzyme XP) increased weight gain and feed intake in broiler chicks. Onyango *et al.* (2004) evaluated the efficacy of three *E. coli* derived phytase preparations on the performance and nutrient utilization of broiler chicks and observed similar weight gain and feed intake. Silverside *et al.* (2004) also compared three phytase enzyme preparations derived from the same *E. coli* gene but produced in *Saccharomyces* *cerevisiae* (A), *Pichia pastoris* (B), and *Pseudomonas fluorescens* (C) with a commercial phytase enzyme preparation added to wheat soyabean meal diets fed to broiler chicks and observed better performance with enzyme B. Pillai *et al.* (2006) observed that increasing iP resulted in a linear increase in weight gain and feed intake and adding *E. coli* phytase resulted in a linear and quadratic increase in weight gain. They also noticed that *E. coli* phytase released more P than the fungal phytases that were tested.

Kemme *et al.* (1997) found that feed intake and ADG were not significantly affected by the addition of phytase (400 PU/kg of diet) to corn-soyabean meal based diet in pigs. Radcliffe *et al.* (1998) observed no effect on performance of crossbred weanling pigs fed a corn-soyabean meal based diet low in Ca and P with different levels of added phytase (0, 250, 500, or 750 PU/kg) and citric acid (0, 1.5, 2 and 3.0 per cent). Moreira *et al.* (2003) also observed that increasing phytase levels from 253, 759, 1265 to 1748 PU/ kg feed did not affect the dietary feed intake and daily weight gain in diets containing defatted rice bran for growing swine. Similarly Park *et al.* (2003) opined that a solid state fermented phytase complex did not affect ADG or average daily feed intake in barrows fed with low aP, corn-soyabean meal diets. Cervantes *et al.* (2004) found no effect on phytase supplementation (500 and 1000 PU/kg diet) on ADG in growing pigs fed basal sorghum-soyabean meal diet.

Martinez *et al.* (2004) observed that the current pharmacological doses of Zn fed to pigs (2000 mg Zn/kg) could be reduced to 1000 mg Zn/kg by adding phytase. Williams *et al.* (2005) on the contrary observed that dietary phytase decreased ADG in pigs fed 1000 or 2000 ppm Zn without affecting average daily feed intake during any period in nursery pigs. However added Zn linearly increased ADG and average daily feed intake in all phases of the experiment.

Akyurek *et al.* (2005) observed that phytase supplementation did not affect body weight gain and feed intake in broilers. No significant effect on average daily feed intake of rabbits supplemented with dietary phytase was reported by Xian *et al.* (2004).

2.2.2 Feed Efficiency

Lei *et al.* (1993) obtained positive results with supplemental phytase for gain: feed ratio in their experiment in pigs fed with a corn–soyabean meal based diet. Cromwell *et al.* (1993) observed decreased efficiency of gain in pigs fed P deficient diets, but on phytase supplementation they restored feed: gain ratio to levels to that fed with adequate P. Adeola *et al.* (1995) also found improved feed efficiency with addition of phytase at 1500 PU/ kg to the diet regardless of level of zinc in pigs. Qian *et al.* (1996) observed a higher gain:feed ratio in pigs fed diets with higher level of phytase and 0.36 per cent tP. Biehl *et al.* (1996) also demonstrated that phytase addition improved feed efficiency in amino acid deficient diets rather than amino acid adequate diet in pigs.

Han *et al.* (1997) in their study to evaluate the feasibility of supplemental phytase in swine diets obtained similar feed efficiency for dietary treatments supplemented with microbial phytase, cereal phytase or iP indicating that supplementation of phytase can completely replace iP in the diet of pigs. Murry *et al.* (1997) observed in a 2x3 factorial experiment with diets containing two levels of P, low (0.58) or adequate (0.95 per cent) supplemented with three levels of microbial phytase at 0, 700, or 1,000 PU/kg of diet, that gain:feed ratio was linearly increased with increasing levels of phytase in pigs fed the low P diets. However, the gain:feed ratio decreased in pigs fed the adequate P diets supplemented with phytase. Liu *et al.* (1997) in a 2x3 factorial trial in barrows with two corn-soyabean meal based diets both containing 0.6 per cent Ca and two levels of P (0.32 per cent tP without added inorganic P and 0.48 per cent tP with added inorganic P) supplemented with phytase at 0, 250 and 500 PU/kg of diet observed that phytase supplementation of the low P diet increased gain:feed ratio linearly with increasing levels of microbial phytase in pigs. Subsequently Liu *et al.* (1998) observed that lowering the dietary Ca:tP ratio from 1.5:1 to 1.0:1 with supplementation of 500 PU/kg phytase linearly increased gain: feed ratio during growing phase but had no effect during finishing phase in pigs. Radcliffe *et al.* (1998) observed that addition of citric acid and phytase to corn-soyabean meal based diet which was low in Ca and P improved feed efficiency and additions of citric acid and phytase had no synergistic effects in crossbred weanling pigs.

Sands *et al.* (2001) conducted a study in pigs with corn-soyabean meal based diets containing HAP corn or normal corn and 0 or 600 units of phytase in a 2x2 factorial experiment and observed better feed efficiency in pigs fed phytase supplemented HAP corn diet when compared to pigs fed normal corn. Park *et al.* (2003) in one of their experiments with 24 barrows noticed that the addition of a solid state fermented phytase complex to low aP, corn- soyabean meal diet increased gain:feed ratio.

Williams *et al.* (2005) conducted experiments with different levels of phytase (0 and 500 PU/kg) and Zn (0, 1,000, or 2,000 ppm) and found that gain:feed ratio was linearly increased by Zn and phytase. Brana *et al.* (2006) also observed a linear increase in gain:feed ratio in nursery and grower pigs subsequent to dietary addition of phytase (10,000 PU/kg) whereas in finisher pigs phytase supplementation increased gain:feed ratio quadratically.

Kemme *et al.* (1997) found that gain:feed ratio was not significantly affected by the addition of phytase in the diet of growing finishing pigs. Similarly O'Quinn *et al.* (1997) observed that supplemental phytase and phosphorus did not affect feed efficiency in their experiment in sorghum-soyabean meal based diets in finishing swine. Moreira *et al.* (2003) also observed that phytase addition did not affect the feed conversion efficiency in swine. No effect of phytase supplementation on feed conversion was also reported in growing pigs fed sorghum-soyabean meal diet by Cervantes *et al.* (2004). Martinez *et al.* (2004) also did not observe any effect on feed efficiency in weaned pigs fed diets supplemented with Zn and phytase.

Boling *et al.* (2001) conducted chick growth assay and found that the protein efficiency ratio (PER) values varied greatly among the different ingredients ranging from 1.4 for corn gluten meal to 4.2 for canola meal. They observed that 1200 PU/kg of phytase addition had no significant effect on PER values for any of the ingredients evaluated, except for casein where there was an increase in PER. Hariharan (2003) also observed no significant increase in feed efficiency and protein efficiency in broiler chicks fed diets with citric acid (3.0 per cent) or phytase (700 U/kg diet).

2.2.3 Digestibility of Nutrients

Mroz *et al.* (1994) observed significant enhancement of apparent digestibility of DM, organic matter and CP in pigs fed corn-tapioca-soyabean meal diet supplemented with microbial phytase (800 PU/kg of diet). Murry *et al.* (1997) observed no phytase level x P level interaction for apparent nutrient digestibility when microbial phytase was added to pearl millet-soyabean meal diet. They noted that adding phytase increased apparent digestibility of DM quadratically in the adequate P diets, CP digestibility linearly in the low P diets and CP digestibility linearly and quadratically in the P adequate diets.

Sands *et al.* (2001) fed pigs corn-soyabean meal based diets containing HAP corn or normal corn with (600 PU/kg) or without phytase and noticed a corn x phytase interaction on DM digestibility. They observed that phytase addition resulted in higher DM digestibility in pigs fed the normal corn diets, whereas the reverse was observed in pigs fed HAP corn diets. Omogbenigun *et al.* (2004) also observed higher apparent ileal digestibility of DM, CP and phytate when supplemented with multi enzyme preparation in weaned pigs than that fed unsupplemented diet. Similarly Johnston *et al.* (2004) observed increased ileal digestibility of various amino acids and DM in pigs fed phytase supplemented at 500 PU/kg of corn-soyabean meal diet containing low Ca and P. Kies *et al.* (2005) observed that 1500 PU of phytase/kg feed increased apparent digestibility of DM, N and fat by 2.0, 1.9 and 1.2 per cent, respectively.

Liao (2005) observed that supplementation of microbial phytase at 500 and 1,000 PU/kg diet containing corn-soyabean meal, wheat-soybeal meal and barley-peas-canola meal did not improve the apparent ileal digestibilities of CP and amino acids whereas significant increase in the apparent ileal digestibilities of CP and amino acids was noted in wheat-soyabean meal-canola meal diet. They further concluded that diets formulated with commonly used feed ingredients did not consistently improve the apparent ileal digestibilities of CP and amino acids for weanling pigs and that the amino acid response factor to microbial phytase supplementation depends on diet composition.

Kies *et al.* (2006) conducted an experiment using weaner pigs fed corn-barley soyabean meal diet with phytase supplemented at 100, 250, 500, 750, 1,500 or 15,000 PU/kg diet or 1.5 g of digestible P/kg diet (positive control) and they observed improved DM digestibility in phytase supplemented diets except the diet with 500 PU/kg. Viswanathan *et al.* (2007) investigated the effect of citric acid and citric acid plus microbial phytase on nutrient utilization in Large White Yorkshire pigs fed corn-soyabean meal based diets and observed that the digestibility coefficient of DM, EE and crude fibre were significantly higher for citric acid and citric acid plus phytase supplemented groups than that of control.

Ravindran *et al.* (1999) observed that addition of microbial phytase (1,200 PU/kg) to broiler diets improved the digestibility of protein and amino acids in all feed stuffs tested but the magnitude of response varied depending on the feed stuff and the amino acid considered. Hariharan (2003) also observed in broiler chicks fed with added citric acid (3.0 per cent) or phytase (700 PU/kg diet) or their combination (1.5 per cent citric acid + 350 PU/kg diet) to low aP diets (0.3 per cent) that the DM retention did not differ significantly between groups. Akyurek *et al.* (2005) in a study noted that phytase supplementation to corn based broiler diets containing low aP registered significant improvement in ileal digestibility of EE and similar CP and DM digestibilites when compared with diets containing 0.45 per cent aP. Juanpere *et al.*

(2005) when studied the effects of microbial phytase and glycosidase enzymes and their interactions, in diets based on corn, wheat or barley in broilers observed that the effects of phytase (500 units of phytase) were only significant for DM digestibility of corn diets and starch digestibility of barley diets. Onyango *et al.* (2005) observed that an evolved *E. coli* phytase improved retention of N and amino acids such as arginine, histidine, threonine, tryptophan, valine, aspartate and proline. They also noticed that apparent ileal digestibilities of DM and N were not affected by phytase supplementation to low P diet in chicks.

No significant effect of dietary P or phytase level on DM digestibility was observed by Harper *et al.* (1997) in the grower phase of swine. O'Quinn *et al.* (1997) also noticed that apparent ileal digestibility of DM, GE and N were not affected by phytase supplementation in swine.

Traylor *et al.* (2001) in one of their study in pigs fed soyabean meal based diets supplemented with various levels of microbial phytase found that inclusion of phytase in the diet had no significant effect on the apparent ileal digestibility of CP and different amino acids. Addition of a solid state fermented phytase complex to low aP, corn-soyabean meal diets did not affect DM digestibility (Park *et al.* 2003). Similarly Cervantes *et al.* (2004) also did not observe any effect of phytase supplementation on the apparent ileal digestibility of CP and amino acids in pigs fed sorghum-soyabean meal diet. Jendza *et al.* (2005) reported that adding phytase to the basal diet without supplemental iP had no effect on apparent digestibility of DM, energy and N. No effect of phytase on the apparent total tract digestibility of CP was reported by Liao *et al.* (2005).

Kemme *et al.* (1997) observed that corn-soyabean meal based diets with (500 PU/kg diet) or without supplemented *Aspergillus niger* phytase, that the addition of phytase to the diet significantly reduced the digestibility of DM in piglets but not in growing-finishing pigs and sows.

Dilger *et al.* (2004) observed that when a new microbial phytase (Phyzyme XP) was added to broiler diet at 500 or 1,000 PU/kg, the apparent ileal digestibility of DM was not influenced. Cowieson and Adeola (2005) investigated the additive effects of xylanase, amylase, protease and phytase in the diets of growing broiler chicks. The birds were fed a corn-soyabean male based negative control (NC) marginal in terms of ME and Ca, a nutritionally adequate positive control (PC) diet and the NC diet supplemented with phytase, a cocktail of xylanase, amylase, and protease (XAP) or a combination of phytase and other enzymes. They observed that digestibility of DM and apparent ileal digestible energy was not affected by supplementation of the NC diet with enzymes.

Anaya *et al.* (2008) observed that the DM digestibility was not affected when four levels of phytase (0, 250, 500, and 1,000 U/kg DM) were added to soyabean meal based commercial dog feed.

2.2.4 Mineral Availability

Lei *et al.* (1993) observed in weanling pigs fed a corn-soyabean meal basal diet supplemented with two levels of phytase (0 and 1350 units/g) and three levels of Zn (0, 30 and 60 mg/kg) that supplemental phytase significantly enhanced retention of Ca and P. They also noted that neither supplemental phytase nor Zn affected Zn retention and supplementing corn-soyabean meal diets with microbial phytase at 1350 units/g feed improved bioavailability of phytate P to weanling pigs. Mroz *et al.* (1994) also observed a significant enhancement of apparent digestibility of Ca and tP by 4.3 and 24.1 percentage units respectively when microbial phytase was supplemented at 800 PU/kg of diet in barrows fed corn-tapioca-soyabean meal diets.

Adeola *et al.* (1995) indicated that daily Zn retention was increased when phytase was added at 1,500 PU/kg to diet regardless of dietary Zn level. They also found that apparent Ca, P and Cu balances were improved with phytase addition, and Zn balance was increased with supplemental Zn and phytase in the diet. These results indicated that growth promoting effect of phytase may be due to an overall increase in the availability of minerals.

Murry (1995) noticed a P x phytase interaction for Ca digestibility and a phytase interaction for P digestibility. He could observe an increased Ca and P digestibility of 14.39 and 30.44 per cent respectively for those pigs fed low P diets with microbial phytase. Similarly Harper and Kornegay (1996) observed enhanced P digestibility with phytase supplementation of low P diet in growing finishing pigs. They noted that P digestibility coefficients were 28.7, 32.6 and 41.3 per cent for diets containing 0, 167, 333 and 500 PU/ kg feed. They concluded that supplemental Natuphos phytase rather than traditional P supplementation resulted in a 22 per cent reduction in fecal P excretion while maintaining grower-finisher pig performance. Harper *et al.* (1997) observed that reducing P level in the diet resulted in a reduction in P digestibility in the grower and finisher phase, but addition of 250 or 500 PU/kg of phytase to the low P diet resulted in a linear improvement in P digestibility in the grower and finisher phases and for the pooled phases.

Han *et al.* (1997) observed that the retention of total ingested P increased by 14 and 19 per cent respectively in pigs supplemented with microbial phytase and iP. Kemme *et al.* (1997) observed that digestibilities of Ca and P were not different between the groups of piglets fed either *ad libitum* or rationed amounts of feed. They also observed that, irrespective of piglet weight, phytase supplementation enhanced the digestibilities of Ca and P by 4.6 and 13.6 percentage units respectively.

Murry *et al.* (1997) noted in nursery pigs fed pearl millet-soyabean meal based diets that increasing levels of microbial phytase from 0 to 1000 PU/kg diet to low P (0.58 per cent) and adequate P diets (0.95 per cent) increased Ca and P absorption and retention. They also observed no microbial phytase level x P level interactions for apparent nutrient digestibility.

Liu *et al.* (1997) found that supplemental phytase at 0, 250 or 500 PU/kg of diet linearly increased Ca absorption in barrows fed low P (0.32 per cent) corn-soyabean meal diet than those fed diets containing 0.48 per cent tP. They also noticed that apparent P absorption was increased linearly with graded levels of phytase. They further noted a soaking x phytase interaction for apparent phosphorus absorption. O'Quinn *et al* (1997) could notice that supplemented phytase at increasing dietary levels at 0, 300 or 500 PU/kg of diet reduced fecal P excretions, but increased urinary P excretions. They also observed that ileal and total tract digestibilities of Ca and P increased with increasing phytase supplementation. The net results of the study revealed linear and quadratic improvements in P retention as a result of phytase supplementation.

Liu *et al.* (1998) observed that pig performance and P utilization were increased by phytase supplementation at 500 PU/kg along with lowering Ca:tP ratio from 1.5:1 to 1.0:1. They also found that lowering the Ca:tP ratio linearly decreased Ca intake, apparent Ca absorption but quadratically increased the apparent percentage of Ca digestibility with phytase supplementation. Liu *et al.* (2000) on the other hand found that lowering the dietary Ca:tP ratio in the diets containing phytase linearly increased the apparent absorption of P in the small intestine but Ca absorption was not affected. They also observed that pigs fed the low P diet with a Ca: tP ratio of 1.0:1 had an apparent absorption of Ca or P similar to that of pigs fed the control diet adequate in Ca and P and lowering the dietary Ca:tP ratio to 1.0:1 in a low P diet containing phytase increased the apparent absorption of P in the small intestine. Matsui *et al.* (2000) in an experiment in growing pigs fed corn-soyabean meal based diets containing two levels of P, adequate (0.34 per cent nonphytate P) or a low (0.20 per cent non phytate P) diet supplemented with 1000, 2000 or 4000 PU /kg of yeast phytase and 1000 PU/kg *Aspergillus niger* phytase observed similar results with 1,000 PU/kg *Asperillus niger* phytase and 4,000 PU/kg yeast phytase supplementation both improving bioavailability of P in the diet for growing pigs. They concluded that supplementation of swine diets with yeast phytase was beneficial but its efficacy was less than that of *Aspergillus niger* phytase.

Sands *et al.* (2001) could not notice any phytase x corn interaction on Ca balance in pigs fed HAP corn based diet with 600 PU/ kg phytase or without phytase. They further found that bioavailability and balance of P in HAP corn was superior to that of normal corn based diet. Veum *et al.* (2002) observed that the phosphorus in low phytate barley was about two times more available than the phosphorus in normal barley.

Gentile *et al.* (2003) in a study on new biosynthetic, heat-stable enzyme derived from the sequences of multiple homologous phytases in improving dietary phytate phosphorus utilization by weanling pigs concluded that the new phytase was effective in improving the bioavailability of phytate P of corn soyabean meal diets in weanling pigs. Park *et al* (2003) also in one of their experiments observed that addition of a solid state fermented phytase at 500 PU/kg to low aP, corn-soyabean meal diets increased digestibility of P resulting in a 10 per cent reduction in P excretion in pigs. Sauer *et al.* (2003) found that supplementation of phytase to the barley-canola meal and the barley-soyabean meal diets in growing pigs increased digestibility and retention of P, as well as digestibility of Ca but no further effect was observed with the supplementation with the mixture of xylanase and ß-glucanase.

Johnston *et al.* (2004) in their study in pigs found that reducing the Ca and P in the diet increased the ileal digestibility of Ca and P and phytase supplementation increased the ileal digestibility of DM, starch, Ca and P. It was also noticed that phytase supplementation also increased ileal digestibility of NDF, but only in diets with adequate levels of Ca and P. Omogbenigun *et al.* (2004) investigated the effect of multienzyme preparations on nutrient digestibility, growth performance and P utilization and excretion in weaned pigs and reported higher total tract P digestibility in enzyme supplemented pigs than control animal.

Jendza *et al.* (2005) observed that exogenous phytase supplementation of starter diets resulted in a linear increase in Ca and P absorption. Kies *et al.* (2005) observed that the apparent absorption of ash and minerals such as Ca, P, Na and K was higher in piglets administered phytase at 1500 PU/kg feed. They also observed that phytase addition increased apparent P digestibility from 52 to 70 per cent. Subsequently Kies *et al.* (2006) observed that in weaner pigs digestibility of ash, P, Mg, Na, K and Cu was increased with increasing phytase levels from 100, 250, 500, 750, 1,500 to 15,000 PU/kg in digestible P deficient corn-barley soyabean meal diets.

Brana *et al.* (2006) observed that there was no effect of Ca:aP ratio or Natuphos enzyme level on the quantity of Ca absorbed in pigs in a comparative study between the two enzymes Natuphos and Phyzyme. However they noted that Ca absorption increased in response to Phyzyme additions. They also found that digestibility coefficients and absorbed P values increased linearly as phytase was added to the negative control diet. Furthermore, P digestion coefficients for pigs fed with phytase supplemented diets were similar or even greater than those fed with adequate P control diet. They further concluded that phytase supplementation improved the apparent digestibility of P over the control by 44 per cent and 22 per cent with Phyzyme and Natuphos, respectively.

Microbial phytase supplementation (1000 PU/kg diet) increased apparent digestibility of P but retention of P was similar in pigs fed with three P adequate diets containing barley, soyabean meal or their mixture (Patras *et al.* 2006). Lyberg . . (2006) noted that digestibility of P was not significantly improved by soaking. Nyachoti *et al.* (2006) observed that supplementing barley based diets with enzyme containing β glucanase and phytase increased the daily P retention in growing pigs. Veum *et al.* (2006) observed that pigs fed barley-based diets containing low P supplemented with 2,500 or 12,500 PU/kg of *E. coli* phytase had greater apparent absorption (per cent) of P, Ca, and Mg than pigs fed the positive control diets. They further noted that addition of E.coli phytase did not increase the apparent percentage absorption of Zn, Fe or Cu.

Viswanathan *et al.* (2007) observed a higher apparent digestibility of Ca and P in both citric acid and citric acid plus phytase supplemented diets in Large White

Yorkshire pigs and they could also note a better apparent digestibility of Mg and Cu in citric acid supplemented groups and of Zn and Mn in citric acid plus phytase supplemented groups.

Rodriguez *et al.* (2002) found that a combination of 600 PU/kg phytase and two per cent citric acid increased egg weight and reduced P content in excreta by more than 50 per cent and reduced nitrogen in excreta in laying hen. They also found that use of phytase alone increased Ca content in yolk and albumin while decreasing that of shell. Silversides*et al.* (2004) compared three experimental phytase enzyme preparations derived from the same *E. coli* gene but produced in *Saccharomyces cerevisiae* (A), *Pichia pastoris* (B) and *Pseudomonas fluorescens* (C) with a commercial phytase enzyme preparation and observed better Ca and P digestibility by enzyme B in broiler chicks.

Xian *et al.* (2004) observed in rabbits that the dietary phytase remarkably reduced the excretion of total P and also excretion of Ca.

Akyurek *et al* (2005) in a study on effect of microbial phytase on growth and nutrient digestibility in broilers could observe that phytase supplementation at 0.5 g/kg to broiler diets significantly increased Ca and P retention.

2.2.5 Blood Parameters

Lei *et al.* (1993) observed that either supplemental phytase (0 and 1350 units/g) or supplemental Zn (0, 30 and 60 mg/kg) increased plasma ALP activity and plasma Zn concentrations in weanling pigs. They further observed that supplemental phytase also significantly enhanced plasma concentrations of iP. Adeola *et al.* (1995) observed that plasma Ca concentration was not significantly affected by dietary phytase in pigs. They further found that though plasma Mg concentration was unaffected by Zn supplementation, it was increased in response to the addition of phytase to the diet. They also noticed that plasma Zn concentration increased when phytase was added to the diet containing no supplemental Zn, but plasma Zn concentration was not affected by phytase, when the diet was supplemented with 100 mg of Zn/kg. Qian *et al.* (1996) investigated the adverse effects of wide Ca:P ratios on supplemental phytase efficacy for weanling pigs fed two dietary P levels and three Ca:tP ratios (1.2:1, 1.6:1, 2.0:1) and observed that serum Ca concentration increased and the P concentration decreased as the Ca:tP ratio increased but Mg, Zn and ALP activity were not influenced by Ca:tP ratio. They also noted that serum Ca:P concentration was affected by phytase supplementation over the 4 week trial, but serum Mg and Zn concentration were not affected by dietary treatment.

Liu *et al.* (1997) observed that phytase supplementation at 0, 250 and 500 PU/kg to corn-soyabean meal diet linearly increased serum iP concentration and total acid phosphatase activity on day 21 and 42 in barrows. They also found a soaking x phytase linear interaction for serum iP and a higher serum iP concentration in pigs fed the phytase supplemented diets with soaking. They further found that phytase or soaking had no effect on serum Ca concentration. O'Quinn *et al.* (1997) also observed that serum P concentration was increased in response to the addition of iP and phytase to sorghum-soyabean meal based diets of finishing swine. Murry *et al.* (1997) added three levels of microbial phytase (0, 700 and 1000 PU/kg of diet) and two levels of P (0.58 and 0.95 per cent) to a pearl millet-soyabean meal diet in nursery pigs and observed a microbial phytase level x P level interaction for serum iP concentration. They observed increased serum Zn concentration in pigs fed supplemental phytase, but no consistent effect of dietary microbial phytase was observed for serum Ca, Mg and Cu concentrations. Han *et al.* (1997) compared the effect of microbial phytase, cereal phytase and iP supplementation to basal diets in pigs from weaning to finishing and noted that pigs fed the basal diet supplemented with microbial phytase or iP had higher serum iP concentrations than pigs of the other two groups. They also noted a higher serum ALP activity in pigs fed control diets than those of the other groups.

Sands *et al.* (2001) found that in pigs fed diets containing HAP corn and normal corn plus phytase plasma iP concentration was increased by both corn and phytase. Johnston *et al.* (2004) observed that phytase addition in diets of pigs increased plasma concentrations of glucose, insulin, urea N and α-amino N. Martinez *et al.* (2004) could observe higher plasma P concentration in pigs fed phytase supplemented diets. They noticed that dietary supplementation of Zn and phytase increased hepatic P but did not affect renal P. They also observed that the dietary Zn and supplemental phytase linearly increased plasma, kidney and liver Zn concentration. Kies *et al.* (2005) observed 20 per cent higher plasma glucose, K and P in phytase fed piglets than in control piglets while plasma concentrations of Na, Cl and Ca were unaffected by the treatment.

Onyango *et al.* (2004) evaluated the efficacy of three *E.coli*-derived phytase preparations on the performance and nutrient utilization of broiler chicks and noticed that all 3 phytase diets outperformed the low P diet in bone mineral content and serum P levels.

Moreira *et al.* (2003) supplemented phytase to diets containing defatted rice bran for growing swine and observed that phytase levels did not affect plasma Ca and P or Ca:P ratio. Jendza *et al.* (2005) fed low P diets supplemented with increasing levels of *E.coli* phytase, resulted in a linear increase in the plasma concentration. Williams *et al.* (2005) conducted three experiments to determine the effects of two levels of phytase (0 and 500 units/kg) and three levels of excess Zn (0, 1000, 2000 ppm) or their combination in diets in a 2 x 3 factorial arrangement in nursery pigs and found that plasma Zn was increased by supplemental Zn but not affected by phytase addition.

Shelton *et al.*(2004) conducted an experiment using diets with reduced levels of Ca and aP without trace mineral premix in growing–finishing swine and noted increased hematocrit value in pigs as a result of phytase supplementation.

2.2.6 Bone and Carcass Characteristics

Harper and Kornegay (1996) in their study in growing–finishing pigs fed diets supplemented with Natuphos phytase at 0, 167, 333 and 500 PU/kg reported the ash content of tenth rib as 32.7, 34.4, 36.7 and 38.7 per cent respectively. They also opined that the carcass evaluation data did not reveal any effects due to phytase supplementation. Similarly Qian *et al.* (1996) noted that when weanling pigs were fed with two dietary P levels (0.36 or 0.45 per cent tP) in combination with three Ca:tP ratios (1.2 :1, 1.6:1, 2.0 :1) along with increasing levels of phytase from 700 to 1050 PU/kg did not improve the bone measurements. They also observed that shear force, stress and energy and percentage of ash of both metacarpal and 10^{th} rib linearly decreased as the Ca:tP ratio became wider and the bone measurements were greater for pigs fed the higher P level. Han *et al.* (1997) found that breaking forces of the metatarsal and metacarpal bones at 50 and 90 kg body weight were similar in pigs fed the basal diet supplemented with microbial phytase and iP. Harper *et al.* (1997) observed that tenth rib mineralization based on shear force and ash were linearly increased as phytase or P was added to the low P diet in pigs fed corn-soyabean meal diets. But they could not find any effect on pork quality such as loin colour, firmness/wetness, marbling, subcutaneous fat depth at any location or in loin muscle area and depth at the 10^{th} rib as a result of P or phytase level.

O'Quinn *et al.* (1997) noticed that length and weight of bones were not affected by supplementation of iP at 0.08 and 0.04 per cent or phytase at 0, 300 or 500 PU per kg diet to sorghum-soyabean meal based diets of finishing swine but there was a quadratic response in the length of 3^{rd} metatarsal bone with increasing phytase supplementation. The quantitative carcass traits were not affected by the addition of iP to the basal diet while phytase supplementation affected dressing percentage and marbling.

Liu *et al.* (1998) in their study observed that lowering the dietary calcium to tP ratio from 1.5:1, 1.3:1 and to 1.0:1 in low P corn-soyabean meal diets supplemented with microbial phytase 500 PU/kg, that there were no linear or quadratic effects of Ca:tP ratio on fresh or fat free dry metacarpal bone weight, while lowering the dietary Ca:tP ratio linearly increased metacarpal bone breaking strength and ash weight. They further observed that lowering the Ca:t P ratio in the low P dietary treatments linearly increased body weight at slaughter and hot carcass weight but similar carcass characteristics in pigs fed diets with a Ca:tP ratio of 1.0:1 and those fed the positive control diet. Landblom *et al.* (2002) also observed similar carcass characteristics among pigs receiving a reduced P diet plus Allozyme phytase and those receiving Fibrozyme. Moreira *et al.* (2003) observed that dietary levels of phytase at 253, 759, 1265 and 1748 PU/ kg did not affect Ca and P ratio of bone ash in growing pigs. They also observed a quadratic relationship between phytase levels and the percentages of P and Ca in bone ash. Kies *et al.* (2005) noticed no difference between the weights of the entire gastrointestinal tract, heart and liver even though the weight of small intestine was numerically lower and weight of pancreas was six per cent lower in phytase supplemented than that of control piglets.

Cromwell *et al.* (1993) observed in pigs fed diets with adequate (0.5 per cent) and inadequate (0.4 or 0.3 per cent) P during growing phase followed by adequate (0.4 per cent) or inadequate (0.3 per cent) P in the finishing phase, that higher levels of phytase improved bone breaking strength. Biehl *et al.* (1996) noted a marked response in ash content of fibula, scapula and metatarsal bones on phytase supplementation (1,200 PU/kg) to a vitamin D3-adequate, P-deficient corn-soyabean meal diet in young pigs. Murry *et al.* (1997) observed no phytase level x P level interaction for the change in bone mineralization in an experiment in gilts fed microbial phytase to a pearl millet soyabean meal based diet on growth. They observed that graded levels of phytase linearly increased bone mineralization in pigs. Radcliffe *et al.* (1998) found linear increase in rib shear force, shear energy, dry bone weight, and ash weight, ash percentage on phytase addition to a corn-soyabean meal based diet low in Ca and P. They also noticed beneficial effect of citric acid addition to weanling pig diets but no synergistic effects were observed. Park *et al.* (2003) noticed increased bone breaking strength and ash percentage with solid state fermented phytase supplementation of corn-soyabean meal diets. The accretion rate of P and ash were also greater for pigs fed diets with solid state fermented phytase.

Omogbenigun *et al.* (2004) also observed improved bone ash content in weaned pigs supplemented with multienzyme preparations. Shelton *et al.* (2004) observed that adding phytase to low calcium and aP diet, improved bone strength and ash per cent in pigs. They also noted that adding phytase to the diet decreased concentration of iron in the bile and Na, Mg and K in the bone. They also found that Zn concentration in the bone was decreased in pigs fed the diet without the trace mineral premix but the effect was much greater in pigs without phytase supplementation. They also noticed better hot carcass weight, dressing per cent and liver and kidney weight as a percentage of final body weight and carcass length with phytase supplementation. Jendza *et al.* (2005) noticed a linear increase in bone ash of the metacarpals with *E.coli* phytase supplementation in starter, grower and finisher pigs.

Brana *et al.* (2006) compared the effectiveness of two phytase enzymes (Phyzyme and Natuphos) in pigs and evaluated fibula ash and could find that bone ash per cent was lower in both the negative control and Natuphos added at 250 PU/kg but it increased linearly with increasing levels of dietary Phyzyme.

Dilger *et al.* (2004) conducted two trials to evaluate the efficacy of a new microbial phytase (Phyzyme XP) for broiler chicks fed corn-soyabean meal based diet and they noted that phytase addition increased tibia and toe ash. Onyango *et al.* (2005) observed that an evolved *E.coli* phytase improved percentage tibia ash in broilers fed corn-soyabean meal based mash diets and they concluded that an evolved *E.coli* phytase improved bone characteristics. Pillai *et al.* (2006) observed that the amount of available P released ranged from 0.12 to 0.24 per cent for *E.coli* phytase, compared with 0.07 to 0.18 per cent from fungal phytase 1 (FP1) and 0.03 to 0.11 per cent from fungal phytase 2 (FP2) at phytase supplementation levels of 250 and 4000 PU/kg respectively and when based on tibia ash weight (mg/chick) P release appeared to be maximized at a level of 1000 PU/kg of *E.coli* phytase. They opined that *E.coli* phytase was effective for maintaining carcass yield characteristics without increasing the incidence of bone breakage.

Augspurger *et al.* (2003) in their trial in chicks found that tibia ash responded quadratically in response to graded levels of ECP up to 1500 PU/kg. They observed phosphorus release values of 0.08 per cent for Natuphos, 0.04 per cent for Ronozyme, and 0.11 per cent for ECP. Augspurger and Baker (2004a) observed in chicks that tibia ash weight was greater for ECP than for FP1 or FP2 with FP1 producing greater responses than FP2. They also observed that supplemental ECP but not FP1 or FP2, resulted in greater tibia ash weight than iP supplementation. Onyango *et al.* (2004) noticed that three *E. coli* derived phytase preparations outperformed the low P diet in bone mineral content, density, strength and ash percentage in broiler chicks.

Pintar *et al.* (2005) conducted a 21-day experiment with day-old broilers to assess the effect of phytase supplementation to cereal-soyabean meal based diets on the mineral content in tibia (Ca, P, Fe, Mg, Cd and Zn) and found that supplemental phytase increased the mineral levels in tibia. A statistically significant increase was observed for Fe and Mg but not for Ca, P, Cd and Zn content in tibia. In the case of Fe and Mg contents, they observed significant cereal x phytase interaction.

Akyurek *et al.* (2005) studied the effect of microbial phytase on growth performance and nutrient digestibility in broilers and found no significant effect on toe DM, crude ash and Ca and P contents of 21 day old broilers and they opined that microbial phytase addition to broiler chick diets could reduce environmental pollution by decreasing P excretion.

Materials and Methods

3. MATERIALS AND METHODS

Two experiments were conducted to study the effect of dietary supplementation of calcium, phosphorus and phytase in different levels on growth, mineral availability and their interactions in cross bred pigs.

3.1 EXPERIMENT 1

A growth study was carried out for a period of 88 days in Large White Yorkshire x Desi piglets to find out the requirement of calcium and phosphorus in their rations and to study the effect of phytase supplementation on the availability of macro and trace minerals.

3.1.1 Experimental Animals

Sixty four weaned Large White Yorkshire x Desi piglets (32 male and 32 female) belonging to the Centre for Pig Production and Research, Mannuthy were used as experimental animals. Male piglets were castrated and all animals were dewormed before the start of the experiment. The piglets were randomly divided into eight groups as uniformly as possible with regard to age, sex and weight. Piglets of each group were allotted randomly into four pens with two piglets in each pen. Two levels of calcium (0.6 and 1 per cent) and two levels of phosphorus (0.3 and 0.6 per cent) were used with phytase (750 units /kg) and without phytase in a 2x2x2 factorial completely randomized design.

3.1.2 Housing And Management

Piglets in each replicate were housed in separate pens in the same shed with facilities for feeding and watering. All animals were maintained under identical management conditions. The animals were washed every day in the morning before 10 AM and stalls were cleaned twice daily before morning and afternoon feeding. Restricted feeding was followed throughout the experimental period by allowing them to consume as much as they could, within a period of one hour and the balance feed was collected and weighed after each feeding. Clean drinking water was provided *ad libitum* in all the pens throughout the experimental period.

3.1.3 Experimental Rations

The animals were fed with standard grower ration with 18 per cent crude protein (CP) and 3200 kcal of metabolisable energy (ME) /kg of feed up to 50 kg body weight and finisher ration with 16 per cent CP and 3200 kcal of ME /kg of feed from 50 to 70 kg body weight as per NRC (1998). Eight dietary treatments with two levels of Ca (0.6 and 1 per cent) and two levels of P (0.3 and 0.6 per cent) were used with (750 units/kg) and without phytase in a 2x2x2 factorial CRD, as given below.

T1- Ration containing 0.6 per cent calcium and 0.3 per cent phosphorus

T2- Ration containing 0.6 per cent calcium, 0.3 per cent phosphorus and 750 units of phytase / kg feed

T3- Ration containing 0.6 per cent calcium and 0.6 per cent phosphorus

T4- Ration containing 0.6 per cent calcium, 0.6 per cent phosphorus and 750 units of phytase / kg feed

T5- Ration containing 1 per cent calcium and 0.3 per cent phosphorus

T6- Ration containing 1 per cent calcium, 0.3 per cent phosphorus and 750 units of phytase / kg feed

T7- Ration containing 1 per cent calcium and 0.6 per cent phosphorus

T8- Ration containing 1 per cent calcium, 0.6 per cent phosphorus and 750 units of phytase / kg feed

The ingredient composition of the starter and finisher rations are given in Tables 1 and 2, respectively.

Table 1. Ingredient composition of starter rations

Ingredient	Starter rations							
	T1	T2	T3	T4	T5	T6	T7	T8
Yellow maize, kg	69.50	69.50	69.50	69.50	69.50	69.50	69.50	69.50
Soyabean meal, kg	28.20	28.20	28.20	28.20	28.20	28.20	28.20	28.20
Salt, kg	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Lysine, kg	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Dicalcium phosphate,kg	1.70	1.70	1.70	1.70	1.70	1.70	1.70	1.70
Total	100	100	100	100	100	100	100	100
To 100 kg of the above mixture added Shell grit, kg	0.60	0.60	0.60	0.60	1.70	1.70	1.70	1.70
Na_2HPO_4, kg	-	-	1.16	1.16	-	-	1.16	1.16
Phytase, g [1]	-	30	-	30	-	30	-	30
Zinc oxide, g	75	75	75	75	75	75	75	75
Indomix, g [2]	25	25	25	25	25	25	25	25
Rovi BE, g [3]	25	25	25	25	25	25	25	25

[1] Maxiphos (Polchem Hygiene laboratories PVT.Ltd, Pune) containing 2500 units of phytase per gram.

[2] Indomix A, B2, D3, K (Nicholas Piramal India Ltd, Mumbai) containing Vitamin A-40,000 IU, VitaminB2-20mg, Vitamin D3-5000 IU, and Vitamin K-50mg, per gram.

[3] Rovi BE (Nicholas Piramal India Ltd, Mumbai) containing Vitamin B1-4mg,Vitamin B6-8mg, Vitamin B12-40mg, Niacin-60mg, Calcium pantothenate 40mg and Vitamin E-40mg, per gram.

Table 2. Ingredient composition of experimental finisher rations

Ingredient	Finisher rations							
	T1	T2	T3	T4	T5	T6	T7	T8
Yellow maize, kg	76.8	76.8	76.8	76.8	76.8	76.8	76.8	76.8
Soyabean meal , kg	21	21	21	21	21	21	21	21
Salt, kg	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Dicalcium phosphate, kg	1.70	1.70	1.70	1.70	1.70	1.70	1.70	1.70
Total	100	100	100	100	100	100	100	100
To 100 kg of the above mixture added Shell grit, kg	0.6	0.6	0.6	0.6	1.7	1.7	1.7	1.7
Na_2HPO_4, kg	-	-	1.16	1.16	-	-	1.16	1.16
Phytase, g [1]	-	30	-	30	-	30	-	30
Zinc oxide, g	75	75	75	75	75	75	75	75
Indomix, g [2]	25	25	25	25	25	25	25	25
Rovi BE, g [3]	25	25	25	25	25	25	25	25

1 Maxiphos (Polchem Hygiene laboratories PVT.Ltd, Pune) containing 2500 units of phytase per gram.

2 Indomix A, B2, D3, K (Nicholas Piramal India Ltd, Mumbai) containing Vitamin A-40,000 IU, Vitamin B2-20mg, Vitamin D3-5000 IU and Vitamin K-50mg per gram.

3 Rovi BE (Nicholas Piramal India Ltd, Mumbai) containing Vitamin B1-4mg, Vitamin B6-8mg, Vitamin B12-40mg, Niacin- 60mg, Calcium pantothenate 40mg and Vitamin E-40mg per gram.

3.1.4 Feeding Trial

The experimental animals of the eight dietary treatments were maintained on their respective feeding regimes during the experimental period. The pigs were weighed at the beginning of the experiment and later on at fortnightly intervals. Daily feed intake was recorded.

3.1.5 Plasma Biochemical Studies

Blood samples were collected at the beginning of the experiment and subsequently at the 12^{th} week of the experiment using heparin as anticoagulant in clean dry test tubes. Blood samples were centrifuged at 3000 rpm for 10 minutes to separate the plasma for analyzing Ca, Mg, Mn, Cu and Zn levels by Atomic Absorption Spectrophotometer (Perkin Elmer 3110) using hollow cathode tubes and phosphorus by Phosphomolybdate method, using the kit supplied by Agappe diagnostics, Shailesh Industrial Complex, Thane. The alkaline phosphatase enzyme activity in plasma samples was estimated using the kit supplied by Agappe diagnostics, Shailesh Industrial Complex, Thane.

3.1.6 Digestion Trial

A digestibility trial was conducted at the end of the experiment to determine the digestibility of nutrients and percentage availability of minerals of the experimental diets by total collection method. Before the commencement of the actual collection period, animals were subjected to a preliminary period of three days when they were fed the same quantity of the feed. Faecal matter was collected for three days as and when they were voided, uncontaminated with feed, dirt or urine. Faecal matter collected daily from each animal was weighed and representative samples were taken after thorough mixing. These samples were placed in double lined polythene bags, labeled and kept in deep freezer until further analysis. The representative samples of feed offered during the collection period were also taken daily and were pooled and sub samples were taken for analysis. The faecal samples and feed samples were analyzed for proximate principles (AOAC, 1990) and minerals such as Ca, Mg, Zn, Cu and Mn were analyzed using Atomic Absorption Spectrophotometer (Perkin Elmer 3110) after wet ashing using nitric acid and perchloric acid (2:1). Phosphorus contents of both feed and faecal samples were analyzed by colorimetry (Vanado-molybdate method, AOAC, 1990) using Spectrophotometer (Spectronic 1001 plus, Milton Roy, USA).

3.1.7 Statistical Analysis

Data collected on various parameters were statistically analyzed by Factorial Completely Randomized Design (CRD) method as described by Snedecor and Cochran (1994). Means were compared by Duncan Multiple Range Test (DMRT) using MSTATC.

3. 2 EXPERIMENT 2

Based on the results of the first experiment, another study was conducted using rations without added minerals, to assess the effect of phytase on availability of minerals in swine ration.

3.2.1 Experimental Animals

Thirty six Large White Yorkshire x Desi weaned piglets (18 males and 18 females) belonging to the Centre for Pig Production and Research, Mannuthy were randomly selected and were divided into three groups, as uniformly as possible with regard to age, sex and weight. Piglets of each group were allotted randomly into six pens with two piglets in each pen. Male piglets were castrated and all animals were dewormed before the start of the experiment. They were randomly allotted to the three experimental treatments. The photographs of the representative experimental animals belonging to the dietary treatments T1, T2 and T3 are given as plates 1, 2 and 3.

Plate 1. Experimental animals-group 1

Plate 2. Experimental animals-group 2

Plate 3. Experimental animals-group 3

Housing And Management

Each replicate was housed in separate pen in the same shed with facilities for feeding and watering. All the animals were maintained under identical management conditions. The animals were washed every day in the morning before 10 AM and stalls were cleaned twice daily before morning and afternoon feeding. Restricted feeding was followed throughout the experimental period. They were allowed to consume as much feed as they could, within a period of one hour. Balance of feed was collected and weighed before the next feeding. Clean drinking water was provided in all the pens for twenty four hours throughout the experimental period.

3.2.3 Experimental Rations

The animals were fed with standard grower ration up to 50 kg body weight and finisher ration from 50 to 70 kg body weight formulated as per NRC (1998), to contain 18 percent CP and 3200 kcal of ME /kg of feed and 16 per cent CP and 3200 kcal of ME /kg of feed, respectively. Based on the results obtained in the first experiment, ration containing 0.6 per cent Ca and 0.3 per cent P was taken as the control ration.

The three experimental rations were

T1-Control ration containing 0.6 per cent calcium and 0.3 per cent phosphorus

T2- Control ration without any mineral supplements and with 750 units of phytase/kg feed

T3- Control ration without phytase and mineral supplementation

The ingredient composition of the starter and finisher rations are furnished in Tables 3 and 4.

Table 3. Ingredient composition of experimental starter rations

Ingredients	Starter rations		
	T1	T2	T3
Yellow maize, kg	70	70	70
Soyabean meal, kg	29.4	29.4	29.4
Salt, kg	0.5	0.5	0.5
Lysine, kg	0.1	0.1	0.1
Total	100	100	100
To 100 kg of the above mixture added Dicalcium phosphate, kg	1.7	-	-
Shell grit, kg	0.6	-	-
Zinc oxide, g	75		
Indomix AB_2D_3, g [1]	25	25	25
Rovi BE, g [2]	25	25	25
Phytase, g [3]	-	30	-

[1] Indomix A, B2, D3, K (Nicholas Piramal India Ltd, Mumbai) containing VitaminA- 40,000 IU, VitaminB2-20mg, Vitamin D3-5000 IU and Vitamin K-50mg, per gram

[2] Rovi BE (Nicholas Piramal India Ltd, Mumbai) containing Vitamin B1-4mg, Vitamin B6-8mg, Vitamin B12-40mg, Niacin-60mg, Calcium pantothenate-40mg, Vitamin E- 40mg, per gram.

[3] Maxiphos (Polchem Hygiene laboratories PVT.Ltd, Pune) containing 2500 units of phytase per gram

Table 4. Ingredient composition of experimental finisher rations

Ingredients	Finisher rations		
	T1	T2	.T3
Yellow maize, kg	76	76	76
Soyabean meal, kg	23.5	23.5	23.5
Salt, kg	0.5	0.5	0.5
Total	100	100	100
To 100 kg of the above mixture added Dicalcium phosphate, kg	1.7	-	-
Shell grit, kg	0.6	-	-
Zinc oxide, g	75		
Indomix AB_2D_3, g[1]	25	25	25
Rovi BE, g[2]	25	25	25
Phytase, g[3]	-	30	-

[1] Indomix A, B2, D3, K (Nicholas Piramal India Ltd, Mumbai) containing Vitamin A- 40,000 IU, VitaminB2-20mg, Vitamin D3-5000 IU and Vitamin K-50mg, per gram

[2] Rovi BE (Nicholas Piramal India Ltd, Mumbai) containing Vitamin B1-4mg, Vitamin B6-8mg, Vitamin B12-40mg, Niacin-60mg, Calcium pantothenate 40mg, Vitamin E- 40mg, per gram.

[3] Maxiphos (Polchem Hygiene laboratories PVT.Ltd, Pune) containing 2500 units of phytase per gram

3.2.4 Feeding Trial

Piglets of the three groups were maintained on the three experimental rations T1, T2 and T3 from weaning till they attained slaughter weight of 70 kg. The pigs were weighed at the beginning of the experiment and at fortnightly intervals thereafter. Daily feed intake was recorded.

3.2.5 Plasma Biochemical Studies

Blood samples were collected at the beginning and 16^{th} week of the experiment using heparin as anticoagulant in clean dry test tubes. Blood samples were centrifuged at 3000 rpm for 10 minutes to separate the plasma for analyzing Ca, Mg, Mn, Cu and Zn by Atomic Absorption Spectrophotometer (Perkin Elmer 3110) using hollow cathode tubes, and phosphorus by Phosphomolybdate method, using the kit supplied by Agappe diagnostics, Shailesh Industrial Complex, Thane. The alkaline phosphatase enzyme activity in plasma samples was estimated using the kit supplied by Agappe diagnostics, Shailesh Industrial Complex, Thane.

3.2.6 Digestion Trial

A digestibility trial was conducted at the end of the experiment to determine the digestibility of nutrients and availability of minerals of the experimental diets. Before the commencement of the actual collection period, animals were subjected to a preliminary period of three days when they were fed the same quantity of the feed. Total quantities of the faeces voided were collected as and when they were voided, uncontaminated with feed, dirt or urine for three days. Samples that were collected on each day from each animal were weighed and representative samples were taken after thorough mixing. These samples were placed in double lined polythene bags, labeled and kept in deep freezer until further analysis. The representative samples of feed offered during the collection period were also taken each day and were pooled and sub samples were taken for the analysis. The feed and faecal samples were analyzed for proximate principles (AOAC, 1990) and minerals such as Ca, Mg, Mn, Cu and Zn were analyzed using Atomic Absorption Spectrophotometer (Perkin Elmer 3110) after wet ashing using nitric acid and perchloric acid (2:1). Phosphorus contents of the feed and faecal samples were analyzed by colorimetry (Vanado-molybdate method, AOAC, 1990) using Spectrophotometer (Spectronic 1001 plus, Milton Roy, USA).

3.2.7. Radiological Examination

X- ray of mandible and femur bones of two animals, selected randomly from each group was done at Department of Veterinary Surgery and Radiology, College of Veterinary and Animal Sciences Mannuthy, to study the effect of the experimental rations on bone development.

3.2.8 Slaughter Data

On attaining the slaughter weight of 70 kg, six male animals each from the three treatment groups were slaughtered at Meat Technology Unit, Mannuthy and data on carcass weight, back fat thickness, loin eye area and weight of the internal organs such as liver, kidney, heart and spleen, were recorded. Dressing percentage and weight of internal organs as percentage of carcass weight were also calculated.

3.2.9 Bone Ash and Bone Minerals

The left tenth rib of all the slaughtered animals was collected and was kept in the freezer for the estimation of bone ash. The adhering connective and muscular tissues were removed by boiling the bone in one per cent solution of sodium hydroxide for about ten minutes. Then the bones were washed thoroughly and were dried in the oven at 100 ^{o} C overnight. The ash contents of the rib bones were estimated (AOAC, 1990) and Ca and P contents of bone ash were determined using Atomic Absorption Spectrophotometer (Perkin Elmer 3110) and by colorimetry (vanado-molybdate method AOAC, 1990) using Spectrophotometer (Spectronic 1001 plus, Milton Roy, USA), respectively.

3.2.10 Histopathology

Samples of kidney were taken from representative animals belonging to all the three treatment groups and histological study was done at Sudharma Laboratories, Thrissur to find out whether there is any gross abnormality.

3.2.11 Statistical Analysis

Data collected on various parameters were statistically analyzed by Completely Randomized Design (CRD) method as described by Snedecor and Cochran (1994). Means were compared by Least Significant Difference (LSD) test.

Results

4. RESULTS

4.1 EXPERIMENT 1

4.1.1 Proximate Composition

The proximate composition of the eight experimental grower and finisher rations are presented in Tables 5 and 7, respectively. The crude protein content of the eight experimental grower rations were between 18.02 and 18.57 per cent while that of finisher ration ranged from 15.57 to 16.90 per cent.

4.1.2 Mineral Composition

The mineral composition of the eight experimental grower and finisher rations are presented in Tables 6 and 8 respectively. The Ca content of the experimental grower ration and finisher rations ranged from 0.62 to 0.67 per cent for T1, T2, T3 and T4 and from 1.02 to 1.08 per cent for T5, T6, T7 and T8. The P content varied between 0.44 and 0.46 per cent for T1, T2, T5 and T6 and from 0.75 to 0.78 per cent for T3, T4, T7 and T8 of both grower and finisher ration.

4.1.3 Body Weight

Data on body weight of pigs reared under the eight dietary treatments, T1, T2, T3, T4, T5, T6, T7 and T8 recorded at fortnightly intervals are presented in Table 9 and are graphically represented in Fig.1. Average final body weight of pigs of the eight dietary treatments was 48.81, 50.31, 49.88, 49.50, 49.44, 49.88, 48.88 and 47.92 kg, respectively.

4.1.4 Average Daily Gain (ADG)

Total body weight gain and ADG of pigs maintained on the eight dietary treatments groups during the experimental period of 88 days are given in Table 10. The average daily body weight gain of pigs under the eight dietary treatments T1, T2, T3, T4, T5, T6, T7 and T8 were 388, 403, 400, 395, 393, 400, 384, and 375 g, respectively and the data is presented in Fig. 2.

4.1.5 Dry Matter Intake (DMI)

Data on total DMI of pigs maintained under the eight dietary treatments T1, T2, T3, T4, T5, T6, T7 and T8 during the experimental period are presented in Table 10 and are graphically represented in Fig.3. Total DMI recorded for the eight treatments were 179.22, 190.31, 186.69, 189.69, 190.16, 187.39, 184.96 and 181.72 kg, respectively.

4.1.6 Feed Conversion Efficiency

The feed conversion efficiency of pigs maintained on dietary treatments T1, T2, T3, T4, T5, T6, T7 and T8 are presented in Table 10 and the data is presented in Fig. 4. Pigs of experimental groups T1, T2, T3, T4, T5, T6, T7 and T8 registered feed conversion efficiencies of 5.25, 5.37, 5.31, 5.47, 5.49, 5.33, 5.48 and 5.51, respectively.

4.1.7 Digestibility of Nutrients

Chemical composition of faeces of pigs fed with different experimental rations is shown in Table 11. Data on per cent digestibility of nutrients is presented in Table 12 and is graphically presented in Fig. 5. The percentage digestibility values ranged from 80.59 to 86.95 for DM, from 78.36 to 81.85 for CP, from 21.37 to 33.83 for ether extract, from 42.13 to 46.40 per cent for crude fibre and from 91.32 and 91.75 per cent for NFE for the eight experimental rations.

4.1.8 Availability of Minerals

Data on the availability of minerals of the eight experimental rations are given in Table 13 and is graphically shown in Fig.6. The percentage availability of Ca of the eight experimental rations T1, T2, T3, T4, T5, T6, T7 and T8 was 33.70, 36.30, 36.03, 29.77, 29.24, 50.45, 32.25 and 41.08 and that of P was 38.93, 44.47, 50.18, 48.80, 31.71, 47.07, 39.33 and 43.31, respectively.

4.1.9 Plasma Biochemical Parameters

Initial plasma biochemical parameters of animals maintained on dietary treatments T1, T2, T3, T4, T5, T6, T7, T8 were 8.8, 9.2, 8.9, 8.9, 9.1, 9.2, 8.5 and 8.5 mg/dl of Ca, 6.0, 5.13, 5.37, 5.1, 6.1, 4.83, 4.5 and 6.2 mg/dl of P, 2.1, 2.2, 1.9, 1.9, 2.1, 2.2, 1.9 and 2.1 mg/dl of Mg, 0.95, 0.85, 0.85, 0.96, 1.0, 1.25, 1.35 and 0.99 ppm of Zn, 1.15, 1.30, 0.99, 1.15, 1.2, 1.2, 1.0 and 1.1 ppm, of Cu and 0.01, 0.01, 0.01, 0.01, 0.02, 0.01, 0.01 and 0.02 ppm, of Mn, respectively. The activity of alkaline phosphatase enzyme was 230, 240, 270, 280, 225, 250, 245, 245 U/L respectively for animals of the groups T1, T2, T3, T4, T5, T6, T7 and T8. Data on biochemical parameters of blood collected on 85th day of the experiment given in Table 14, show 11.0, 11.5, 10.02, 11.94, 10.43, 10.99, 11.80 and 11.92 mg/dl, of Ca, 5.65, 5.11, 5.17, 5.03, 5.68, 5.31, 5.06 and 6.00 mg/dl, of P., 1.91, 1.95, 1.93, 1.92, 1.84, 1.83, 1.95 and 1.86 mg/dl, of Mg, 1.3, 0.95, 1.17, 1.18, 0.98, 1.12, 0.95 and 0.99 ppm of Zn, and 1.12, 1.28, 1.47, 1.57, 1.33, 1.50, 1.53 and 1.72 ppm, of Cu, respectively. Plasma Mn content was 0.01 ppm for pigs of all the treatments, while the activity of alkaline phosphatase enzyme was 334, 355, 298, 337, 317, 364, 325 and 357 U/L respectively for animals of T1, T2, T3, T4, T5, T6, T7 and T8.

4.1.10 Economics

Data on total body weight gain, total feed intake, total feed cost and cost of feed/kg gain of pigs maintained on the eight dietary treatments T1, T2, T3, T4, T5, T6, T7 and T8 are presented in Table 15. The cost of ingredients used for the study was as per the rate contract fixed by the College of Veterinary and Animal Sciences Mannuthy, for the year 2006-07. Phytase was supplied by Polchem Hygiene Laboratories PVT. Ltd, Pune at the rate of Rs.265 /kg. Cost of feed per kg body weight gain of pigs maintained on the eight dietary treatments were Rs.59.15, 60.19, 60.58, 62.77, 62.12, 60.71, 62.69 and 63.53 respectively.

4.2 EXPERIMENT 2

4.2.1 Chemical Composition

The percentage chemical composition of the grower ration is presented in Table 16 and finisher ration in Table 17.

4.2.2 Mineral Composition

The mineral composition of the three grower as well as finisher rations are presented in Tables 16 and 17, respectively. The Ca content of the control grower ration T1 was 0.75g per cent while that of T2 and T3 were 0.2 and 0.19 per cent respectively and the P content of the three grower rations were 0.57, 0.25 and 0.24g per cent respectively for T1, T2 and T3. The Ca content of the control finisher ration was 0.75g per cent and that of T2 and T3 was 0.2 per cent each while the P content of the three finisher rations was 0.56, 0.24 and 0.25g per cent respectively.

4.2.3 Body Weight

Data of body weight of pigs under the three dietary treatments T1, T2 and T3 recorded at fortnightly intervals are presented in Table 18, and are graphically represented in Fig.7. Average final body weight of pigs of the three dietary treatments was 67.79, 69.54, and 64.75kg, respectively. Total body weight gain of animals belonging to groups T1, T2 and T3 were 59.12, 60.58 and 55.66 kg, respectively and the data is shown Table 18.

4.2.4 Average Daily Gain

Data on ADG of pigs maintained under the three dietary treatments T1, T2, and T3 are presented in Table 19 and in Fig.8. Average daily gain of pigs maintained under the three dietary treatments T1, T2 and T3 was 0.51, 0.53 and 0.48 kg respectively.

4.2.5 Dry Matter Intake

Data on total DMI of pigs maintained under the three dietary treatments T1, T2 and T3, during the experimental period are presented in Table 19 and are graphically represented in Fig.9. Total DM intakes recorded were 334.04 334.09 and 322.27 kg respectively for animals of T1, T2 and T3.

4.2.6 Feed Conversion Efficiency

The feed conversion efficiency of pigs of T1,T2 and T3 are presented in Table 19 and the data is presented in Fig. 10. Pigs of group T1, T2 and T3 registered a feed conversion efficiency of 5.65, 5.52 and 5.79, respectively.

4.2.7 Digestibility of Nutrients

Chemical composition of faeces of pigs fed with different experimental diets is shown in Table 20. Data on percentage digestibility of nutrients of experimental rations T1, T2 and T3 are presented in Table 21 and are graphically represented in Fig. 11. The percentage digestibility of the three rations T1, T2 and T3 were 86.11, 87.92 and 87.95 for DM, 83.63, 85.31 and 84.97 for CP, 52.81, 53.89 and 53.89 for EE, 55.49, 57.41 and 55.94 for CF and 92.22, 93.55 and 93.56 for NFE, respectively.

4.2.8 Availability of Minerals

The percentage availability was 52.03, 64.68 and 59.88 for Ca, 50.07, 58.05 and 52.45 for P, 68.15, 64.20 and 62.81 for Mg, 65.77, 64.93 and 61.78 for Zn, 67.60, 70.68 and 71.09 for Cu, and 72.13, 76.96 and 73.16 for Mn for pigs of groups T1, T2 and T3, respectively. The data are shown in Table 22 and depicted in Fig.12.

4.2.9 Plasma Biochemical Parameters

Biochemical parameters of blood from pigs of the three groups collected before the start of the experiment were 10.29, 10.10 and 10.37 mg/dl of Ca, 5.00, 5.03 and 5.01 mg/dl of P, 2.58, 2.89 and 3.10 mg/dl of Mg, 0.74, 0.72 and 0.76 ppm of Zn, 1.02, 1.13 and 1.19 ppm of Cu and 0.01, 0.01 and 0.02 ppm of Mn, respectively. The ALP enzyme activity was 330.67, 339.67 and 314.33 U/L respectively the three treatments T1,T2 and T3. The corresponding values on various plasma biochemical parameters of blood collected at the 16th week of the experiment from pigs maintained on the three experimental rations were 10.96, 10.56 and 10.67 mg/dl of Ca, 5.22, 5.82 and 5.46 mg/dl of P, 3.29, 3.79 and 3.71 mg/dl of Mg 0.66, 0.68 and 0.69 ppm of Zn, 1.65, 1.69 and 1.64 ppm of Cu, 0.02 ppm of Mn for all the three treatments and 609.83, 552.67 and 550.67 U/L of ALP activity respectively. The data is shown in Table 23.

4.2.10 Slaughter Data

Data on dressing percentage, loin eye area, back fat thickness, weight of internal organs such as heart, kidney, spleen, lungs and liver, bone ash percentage, bone Ca percentage and bone P percentage of the pigs maintained on the three dietary treatments are shown in Tables 23 and 24 and represented graphically in Fig.12 to 19. The dressing percentage of pigs belonging to the three treatment groups were 73.76, 70.20 and 70.16, loin eye area were 3.75, 3.74 and 2.92 sq.inches, back fat thickness being 3.40, 3.41 and 3.39, respectively. The data on weight of internal organs were taken. The weight of heart as percentage of carcass weight of the three treatment groups T1, T2 and T3 were same 0.4 per cent. The weight of lungs as per cent of carcass weight was 1.51, 1.48 and 1.50 respectively for the treatments T1, T2 and T3 respectively. The weight of liver as percentage of carcass weight was 2.47, 2.55 and 2.47 respectively for the three treatment groups while that of kidney was 0.49, 0.44 and 0.46 respectively. The weight of spleen of the three treatment groups was 0.25, 0.34 and 0.28 respectively as percentage of carcass weight. The percentage of bone ash was 49.62, 48.66 and 43.69, while the Ca and P percentages were 19.45 and 8.63, 20.78 and 8.81 and 17.01 and 8.05, respectively for animals of T1, T2 and T3. Photograph of left tenth rib of pigs belonging to the dietary treatments T1 and T3 are given as plates 4 and 5

4.2.11 Radiological Examination

X-ray of femur of pigs belonging to the dietary treatments T1, T2 and T3 are presented as plates 6, 7 and 8, respectively and that of mandibles in plates 9, 10 and 11, respectively.

4.2.12 Histology of kidney

Plates 12, 13 and 14 shows histology of kidney samples collected from animals belonging to the dietary treatments T1, T2 and T3 respectively.

4. 2. 13 Economics

Data on total body weight gain, total feed intake, and cost of feed per kg body weight gain of pigs maintained on the three dietary treatments are presented in Table 25. The cost of ingredients used for the study was as per the rate contract fixed by the College of Veterinary and Animal Sciences Mannuthy, for the year 2006-07. Phytase was supplied by Polchem Hygiene Laboratories PVT. Ltd, Pune at the rate of Rs.265 /kg. Cost of feed per kg body weight gain of pigs maintained on the three dietary treatments were 65.73, 59.90 and 62.44 respectively and is shown in Fig. 20.

Table 5. Proximate composition * of grower rations of experiment 1, %

Parameter	Treatments							
	T1	T2	T3	T4	T5	T6	T7	T8
Dry matter	86.40	87.20	85.78	87.06	87.58	86.01	84.85	86.48
Crude protein	18.51	18.5	18.14	18.5	18.02	18.14	18.57	18.09
Ether extract	1.29	1.53	1.81	1.32	1.53	1.35	1.52	1.46
Crude fibre	1.65	1.22	1.55	1.62	1.85	1.2	1.4	1.99
Total ash	4.85	5.37	5.09	6.05	5.95	6.08	5.56	6.1
Nitrogen free extract	73.70	73.38	73.41	72.51	72.65	73.23	72.95	72.36
Acid insoluble ash	0.64	1.13	0.80	0.77	0.88	0.76	0.51	0.69

* On DM basis

Table 6. Mineral composition * of grower rations of experiment 1

Mineral	Treatments							
	T1	T2	T3	T4	T5	T6	T7	T8
Calcium, %	0.67	0.66	0.64	0.63	1.04	1.02	1.02	1.07
Phosphorus, %	0.46	0.46	0.78	0.76	0.46	0.45	0.76	0.76
Magnesium,%	0.32	0.32	0.31	0.31	0.31	0.31	0.31	0.31
Zinc, ppm	346.72	344.81	335.99	344.01	344.37	353.19	344.73	344.82
Copper, ppm	8.86	8.78	7.95	8.72	7.87	9.52	7.73	7.77
Manganese, ppm	14.77	14.65	13.92	14.53	13.78	15.24	13.53	13.6

* On DM basis

Table 7. Proximate composition * of finisher rations of experiment 1, %

Parameter	Treatments							
	T1	T2	T3	T4	T5	T6	T7	T8
Dry matter	88.90	89.04	88.50	88.19	88.61	87.56	87.26	87.00
Crude protein	16.90	16.22	16.71	15.67	15.59	15.57	16.54	16.33
Ether extract	1.22	1.33	1.18	1.09	1.37	1.48	1.15	1.03
Crude fibre	3.20	3.35	3.17	3.19	2.75	2.65	3.14	3.14
Total ash	5.24	6.16	5.80	6.65	6.47	6.49	6.6	6.98
Nitrogen free extract	73.44	72.94	73.14	73.40	73.82	73.81	72.57	72.52
Acid insoluble ash	0.95	1.53	1.14	1.29	1.18	1.30	1.35	1.58

* On DM basis

Table 8. Mineral composition *of finisher rations of experiment 1

Mineral	Treatments							
	T1	T2	T3	T4	T5	T6	T7	T8
Calcium, %	0.62	0.64	0.64	0.62	1.04	1.08	1.07	1.04
Phosphorus, %	0.44	0.45	0.77	0.78	0.46	0.46	0.77	0.75
Magnesium, %	0.34	0.31	0.34	0.36	0.35	0.37	0.36	0.38
Zinc, ppm	376.23	381.56	390.34	351.17	390.65	398.93	369.28	387.9
Copper, ppm	7.45	7.65	7.85	7.96	7.87	7.92	7.90	7.72
Manganese, ppm	13.94	13.39	13.75	13.93	13.78	13.86	13.83	13.51

* On DM basis

Table 9. Fortnightly body weight of experimental animals maintained on eight dietary treatments in experiment 1.[1]

Treatments	Fortnights					
	1	2	3	4	5	6
T1	14.66	20.94	27.31	32.56	39.88	48.81
T2	14.85	21.44	27.88	33.06	40.81	50.31
T3	14.72	20.71	28.13	33.25	40.74	49.88
T4	14.78	20.88	28.13	32.81	41	49.50
T5	14.82	20.94	28.06	32.19	41.44	49.44
T6	14.72	20.69	27.81	33.19	41.19	49.88
T7	15.1	20.56	28	32.63	40.75	48.88
T8	14.94	20.75	28	32.75	41	47.92
Pooled SE	0.05	0.10	0.10	0.13	0.16	0.27
P	0.99	0.99	0.99	0.99	0.99	0.99

[1] Mean of four values

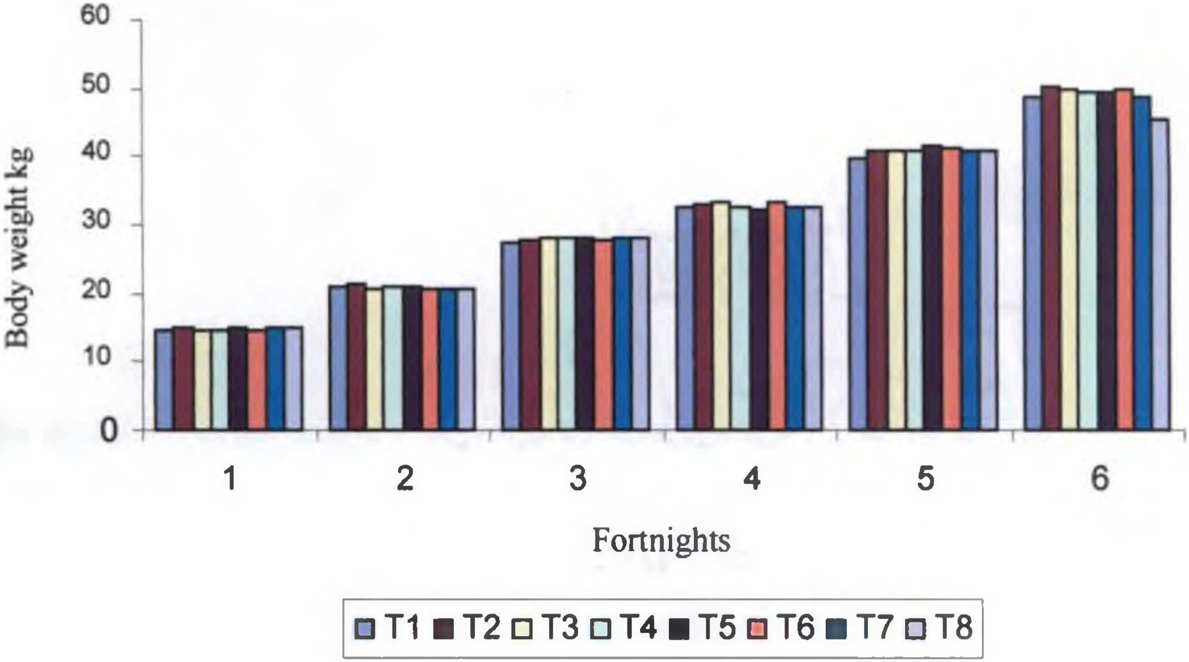

Fig.1. Fortnightly body weight of pigs maintained on eight experimental rations of experiment 1

Table 10. Average daily gain and feed efficiency of experimental animals maintained on eight dietary treatments of experiment 1 [1]

Treatments	Average initial body weight, kg	Average final body weight, kg	Total body weight gain, kg	Average daily gain, g	Total dry matter intake, kg	Feed conversion efficiency
T1	14.66	48.81	34.15	388	179.22	5.25
T2	14.85	50.31	35.46	403	190.31	5.37
T3	14.72	49.88	35.16	400	186.69	5.31
T4	14.78	49.50	34.72	395	189.69	5.47
T5	14.82	49.44	34.62	393	190.16	5.49
T6	14.72	49.88	35.16	400	187.39	5.33
T7	15.10	48.88	33.78	384	184.96	5.48
T8	14.94	47.92	32.98	375	181.72	5.51
Pooled SE	0.05	0.27	0.29	3.35	1.44	0.04
Dietary Ca	NS	NS	NS	NS	NS	NS
Dietary P	NS	NS	NS	NS	NS	NS
Phytase	NS	NS	NS	NS	NS	NS
Ca x P	NS	NS	NS	NS	*	NS
P x phytase	NS	NS	NS	NS	NS	NS
Ca x phytase	NS	NS	NS	NS	**	NS
Ca x P x phyatse	NS	NS	NS	NS	NS	NS

[1] Mean of 4 values
NS- Nonsignificant
* Significant ($P < 0.05$) **($P < 0.01$)

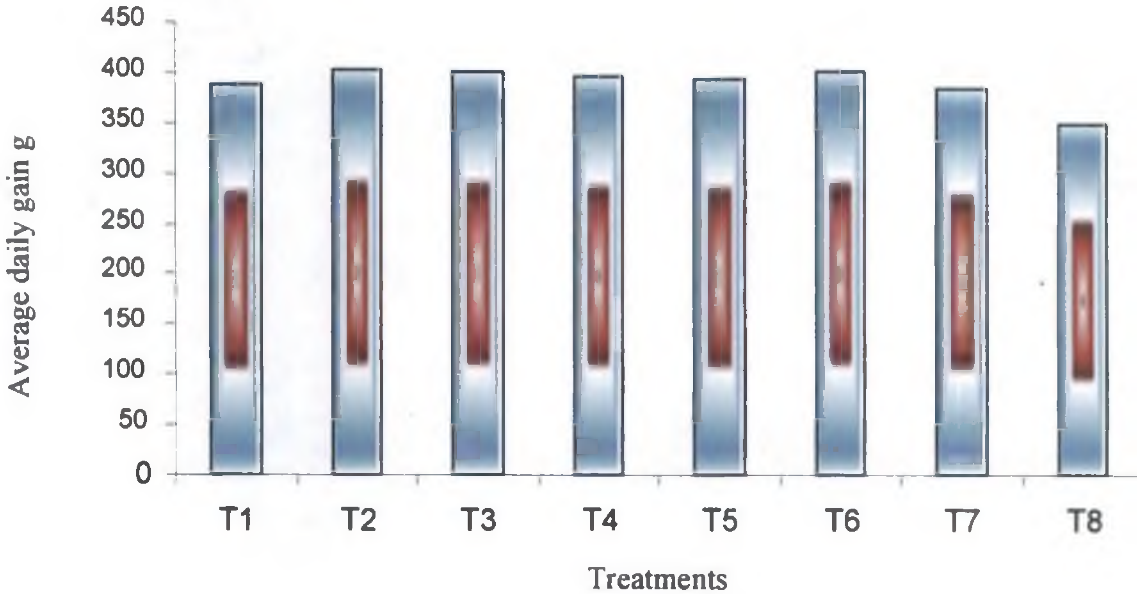

Fig.2. Average daily gain of pigs maintained on eight experimental rations of experiment 1

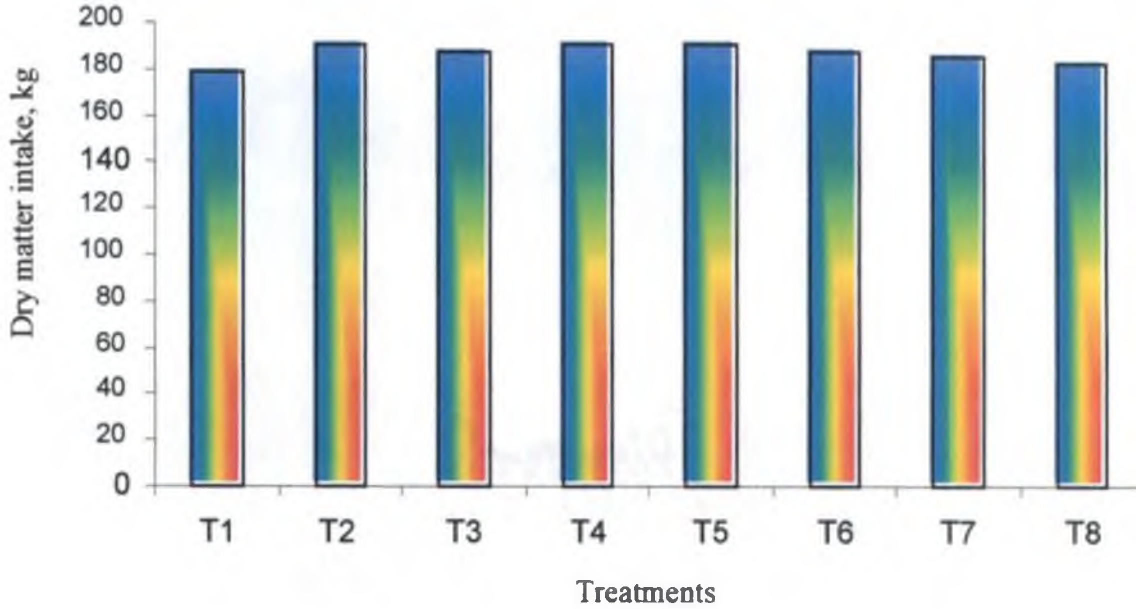

Fig.3. Total dry matter intake of pigs maintained on eight experimental diets of experiment 1

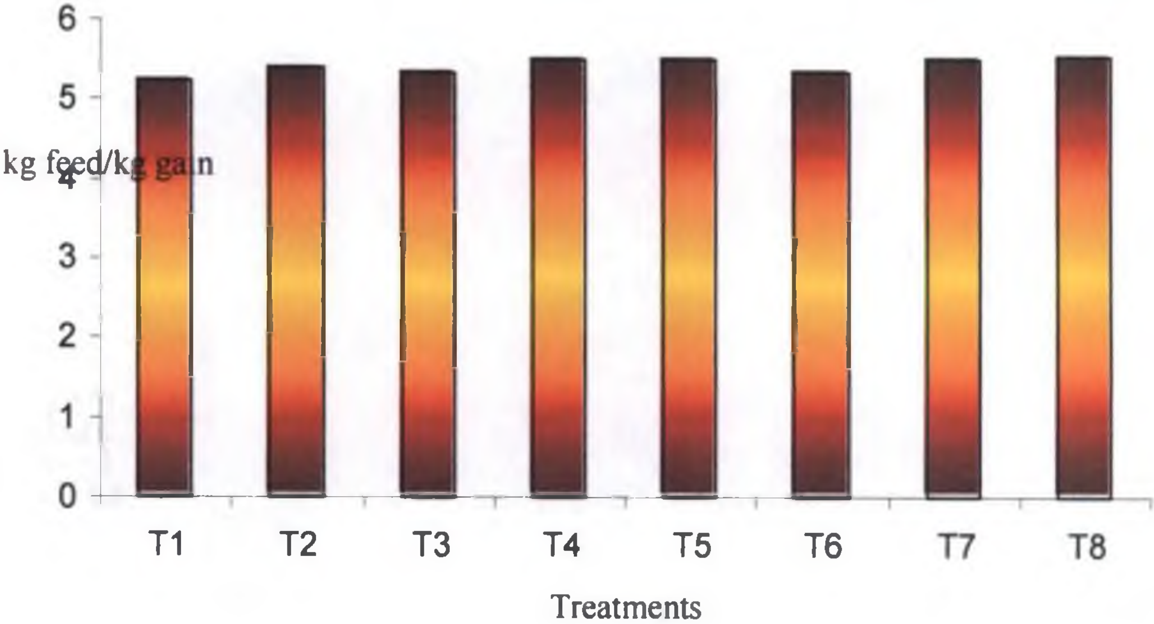

Fig.4. Feed efficiency of pigs maintained on eight experimental rations of experiment 1

Table 11. Chemical composition of faecal samples [*]of experimental animals, maintained on eight dietary treatments of experiment 1

Parameter	Treatments							
	T1	T2	T3	T4	T5	T6	T7	T8
Dry matter, %	73.57	69.76	71.27	72.10	72.31	71.67	69.87	70.92
Crude protein,%	24.45	18.21	22.19	21.20	20.49	20.60	16.51	21.61
Ether extract, %	5.93	5.90	5.08	5.28	5.97	5.83	4.62	4.94
Crude fibre, %	10.99	12.02	9.48	10.42	10.17	9.95	9.24	10.29
Total ash, %	21.45	24.58	23.22	25.23	24.87	26.74	26.52	26.81
Nitrogen free extract, %	37.18	39.29	40.03	37.87	38.50	36.88	43.11	36.35
Acid insoluble ash, %	3.18	8.55	4.55	5.82	4.30	5.08	5.74	4.88
Calcium, %	3.20	2.52	2.74	2.88	4.55	3.66	3.74	3.78
Phosphorus, %	2.03	1.54	2.69	2.62	1.93	1.67	2.42	2.61
Magnesium, %	0.76	0.64	0.89	0.77	0.81	0.80	0.64	0.78
Zinc, ppm	840.49	778.99	801.91	788.16	791.20	765.15	583.99	754.96
Copper, ppm	20.56	16.84	16.29	12.73	15.31	17.92	15.98	14.67
Manganese, ppm	28.31	30.89	22.18	31.61	24.67	29.03	26.06	24.10

* On DM basis

Table 12. Digestibility of nutrients[1] of eight experimental rations, %, in experiment 1

Treatments	Dry matter	Crude protein	Ether extract	Crude fibre	Nitrogen free extract
T1	86.95	81.18	33.83	45.07	93.38
T2	83.72	81.85	28.60	42.13	92.65
T3	84.16	79.00	32.50	42.95	91.32
T4	84.85	79.27	26.37	51	92.2
T5	83.88	78.93	30.13	43.80	91.64
T6	85.29	80.73	37.33	45.43	92.57
T7	80.59	80.62	21.90	43.17	92.44
T8	83.61	78.36	21.37	46.40	91.75
Pooled SE	0.80	1.41	2.28	1.63	0.23
Dietary Ca	*	NS	NS	NS	NS
Dietary P	**	NS	**	NS	NS
Phytase	NS	NS	NS	*	NS
Ca x P	NS	NS	**	NS	NS
P x phytase	*	NS	NS	*	NS
Ca x phytase	**	NS	*	NS	NS
Ca x P x phyatse	NS	NS	NS	NS	NS

[1] Mean of 4 values
NS- Nonsignificant
*Significant (P< 0.05) **(P < 0.01)

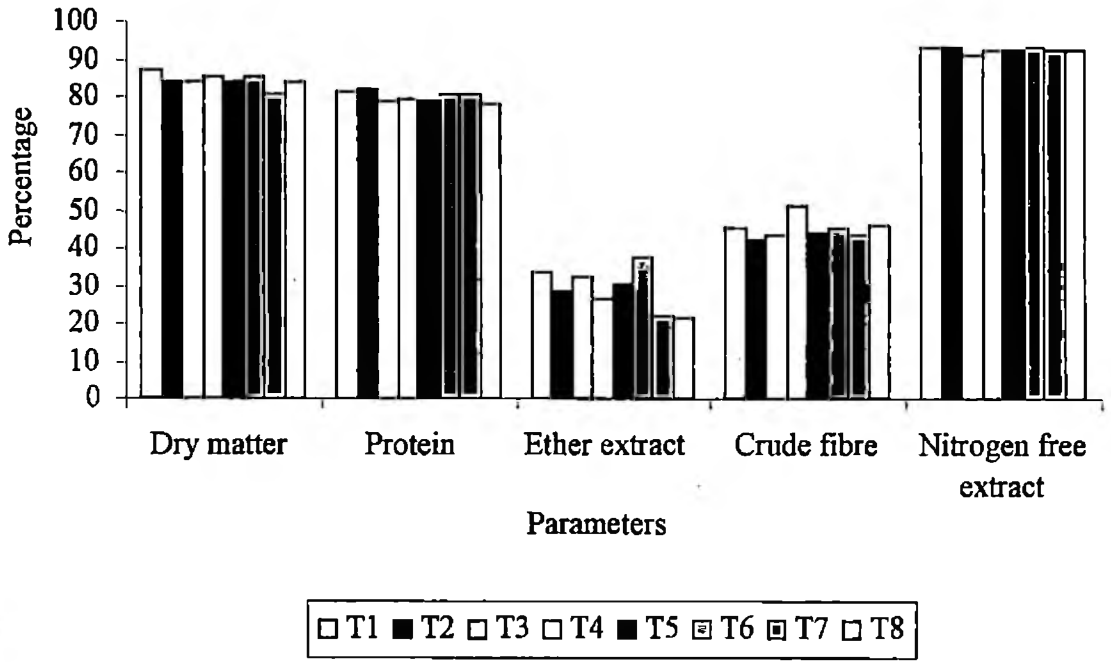

Fig.5. Digestibility of nutrients of eight experimental rations of experiment 1

Table13. Availability of minerals[1] of eight experimental rations, %, in experiment 1

Treatments	Calcium	Phosphorus	Magnesium	Zinc	Manganese	Copper
T1	33.80	38.93	71.06	70.70	73.47	64.18
T2	36.30	44.47	66.90	66.94	62.85	64.46
T3	36.03	50.18	65.42	71.68	75.61	67.97
T4	29.77	48.80	68.27	66.08	65.93	75.94
T5	29.24	31.71	62.85	67.33	71.11	68.46
T6	0.45	47.07	68.05	71.46	68.90	67.09
T7	32.25	39.33	65.51	69.36	63.62	60.41
T8	41.08	43.31	66.92	67.98	70.75	69.03
Pooled SE	2.98	5.04	3.39	2.86	3.29	3.46
Dietary Ca	NS	NS	NS	NS	NS	NS
Dietary P	NS	NS	NS	NS	NS	NS
Phytase	**	NS	NS	NS	NS	NS
Ca x P	NS	NS	NS	NS	NS	*
P x phytase	*	NS	NS	NS	NS	NS
Ca x phytase	**	NS	NS	NS	*	NS
Ca x P x phyatse	NS	NS	NS	NS	NS	NS

[1] Mean of 4 values
NS- Nonsignificant
Significant *($P < 0.05$) **($P < 0.01$)

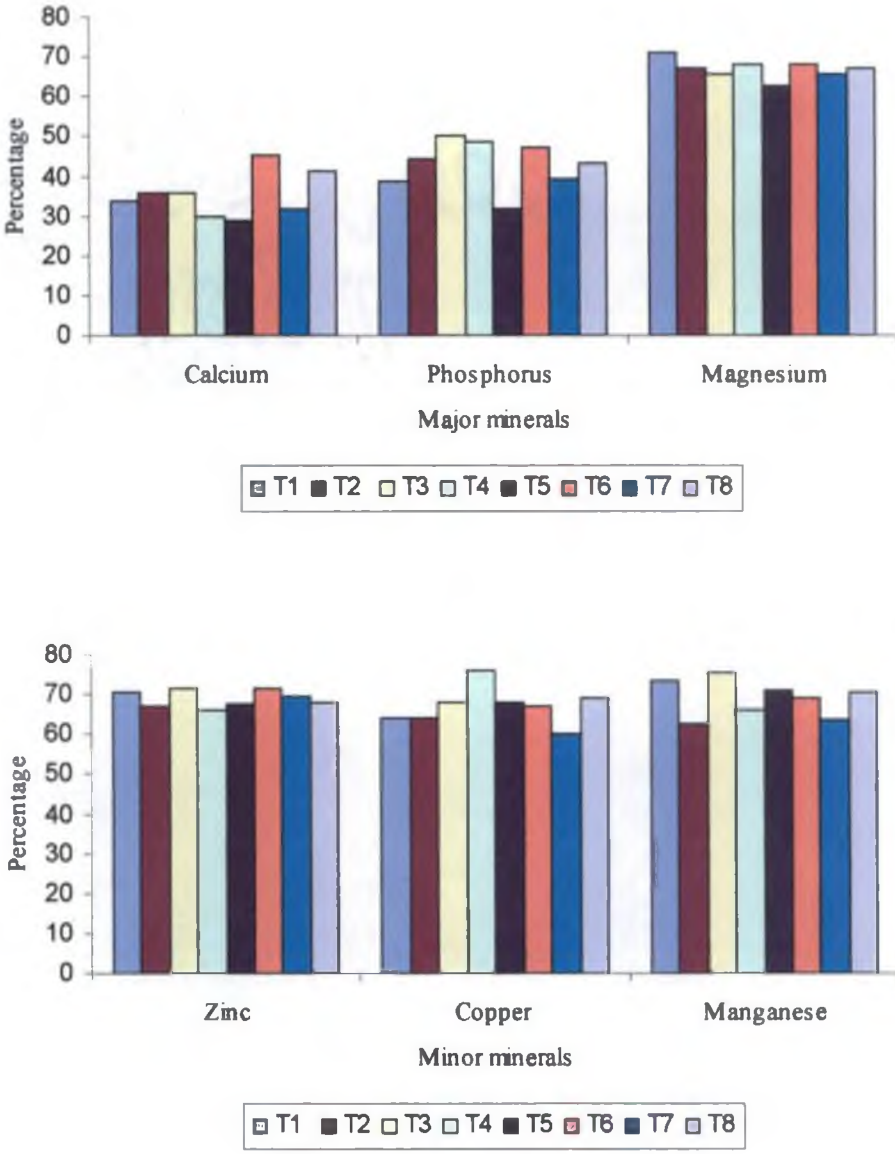

Fig.6. Availability of minerals of eight experimental rations, experiment 1

Table 14. Plasma biochemical parameters of animals maintained on eight experimental rations [1] of experiment 1

Treatments	Calcium mg/dl	Phosphorus mg/dl	Magnesium mg/dl	Zinc ppm	Copper ppm	Mn ppm	Alkaline phosphatase U/L
T1	11	5.65	1.91	1.30	1.12	0.01	230
T2	11.50	5.11	1.95	0.95	1.28	0.01	240
T3	10.02	5.17	1.93	1.17	1.47	0.01	220
T4	11.94	5.03	1.92	1.18	1.57	0.01	218
T5	10.43	5.68	1.84	0.98	1.33	0.01	221
T6	10.99	5.31	1.83	1.12	1.50	0.01	250
T7	11.80	5.06	1.95	0.95	1.53	0.01	215
T8	11.92	6	1.86	0.99	1.72	0.01	245
Pooled SE	0.31	0.04	0.04	0.07	0.07	0.00	3.26
Dietary Ca	NS	**	NS	**	**	NS	**
Dietary P	NS	**	NS	NS	**	NS	**
Phytase	**	NS	NS	NS	**	NS	**
Ca x P	*	**	NS	**	NS	NS	**
P x phytase	NS	**	NS	**	NS	NS	NS
Ca x phytase	NS	**	NS	*	NS	NS	P=0.0524
Ca x P x phyatse	NS	**	NS	**	NS	NS	**

[1] Mean of four values

NS - Non significant (P>0.05), *Significant (P<0.05) ** (P<0.01)

Table 15. Cost of feed per kilogram body weight gain of experimental animals maintained on eight dietary treatments of experiment 1

Parameters	T1	T2	T3	T4	T5	T6	T7	T8
Total body weight gain	34.15	35.46	35.16	34.72	34.62	35.16	33.78	32.98
Total feed intake	179.22	190.31	186.69	189.69	190.16	187.39	184.96	181.72
Cost /kg feed, Rs.	11.27	11.35	11.41	11.49	11.31	11.39	11.45	11.53
Total feed cost, Rs.	2019.81	2160.02	2130.13	2179.54	2150.71	2134.37	2117.79	2095.23
Cost of feed / kg gain, Rs.	59.15	60.91	60.58	62.77	62.12	60.71	62.69	63.53

Table 16. Chemical composition of grower[1] rations of experiment 2, %

Parameter	Treatments		
	T1	T2	T3
Dry matter, %	92.80	92.30	92.24
Crude protein, %	18.43	18.48	18.37
Ether extract, %	2.8	2.54	2.58
Crude fibre, %	3.57	3.23	3.14
Total ash, %	6.35	5.19	4.82
Nitrogen free extract, %	68.85	70.56	71.09
Acid insoluble ash, %	1.74	1.22	0.97
Calcium, %	0.75	0.2	0.19
Phosphorus, %	0.57	0.25	0.24
Magnesium, %	0.33	0.24	0.24
Zinc, ppm	262.03	36.39	37.81
Copper, ppm	9.59	9.52	9.03
Manganese, ppm	13.79	13.44	12.98

[1] On DM basis

Table 17. Chemical composition of finisher [1] rations of experiment 2, %

Parameter	Treatments		
	T1	T2	T3
Dry matter, %	88.85	88.53	87.83
Crude protein , %	16.15	16.32	16.63
Ether extract , %	2.58	2.79	2.64
Crude fibre , %	3.49	3.48	3.32
Total ash, %	5.7	3.42	3.30
Nitrogen free extract, %	71.68	73.99	74.11
Acid insoluble ash, %	0.76	0.66	0.57
Calcium, %	0.75	0.20	0.20
Phosphorus, %	0.56	0.26	0.25
Magnesium, %	0.34	0.24	0.25
Zinc, ppm	336.47	44.18	42.90
Copper, ppm	9.40	9.05	9.80
Manganese, ppm	13.16	13.58	12.44

[1] On DM basis

Table 18. Fortnightly body weights[1] of experimental animals maintained on three experimental rations in experiment 2

Treatment	Fortnights									
	0	1	2	3	4	5	6	7	8	9
T1	8.71	13.00	20.42	27.71	35.33[a]	42.65	49.88[ab]	60.16[ab]	64.92[ab]	67.79[ab]
T2	9.02	13.15	20.38	28.23	37.13[b]	43.73	52.29[a]	62.27[a]	66.71[a]	69.54[a]
T3	9.09	13.42	19.96	27.78	34.21[a]	41.23	48.05[b]	57.84[b]	61.34[b]	64.75[b]
Pooled SE	0.13	0.15	0.18	0.27	0.43	0.96	0.69	0.77	0.87	0.87
P	NS	NS	NS	NS	0.01	NS	0.03	0.05	0.03	0.07

[1] Mean of six values

a, b Means with different superscripts within each column differ ($p<0.05$)

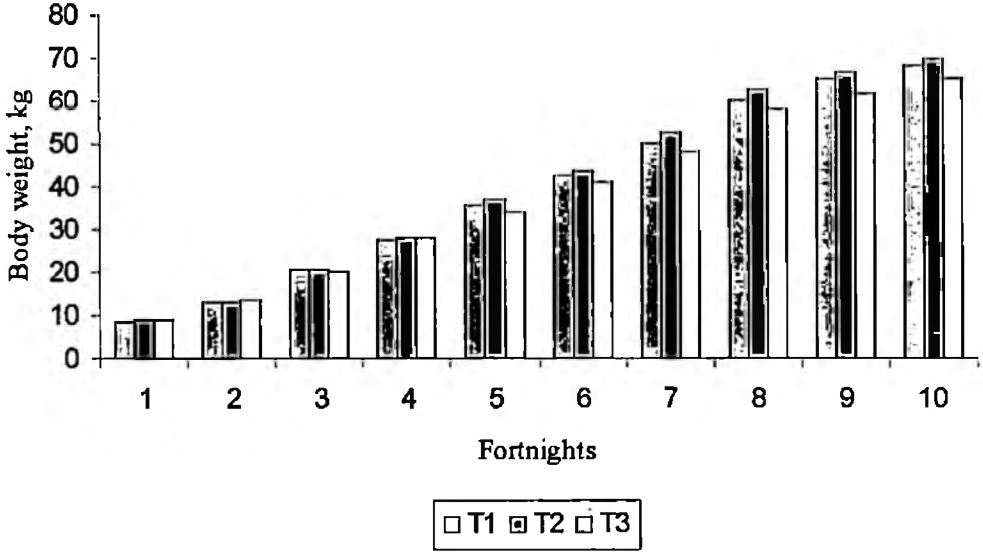

Fig.7. Fortnightly body weight of pigs maintained on three experimental rations, experiment 2

Table 19. Average daily gain[1] and feed efficiency of experimental animals maintained on three experimental rations of experiment 2

Treatment	Average initial weight (kg)	Average final weight (kg)	Total weight gain (kg)	Average daily gain (kg)	Average dry matter intake (kg)	Feed efficiency
T1	8.71	67.79	59.08	0.51 [ab]	334.04	5.65
T2	9.02	69.54	60.52	0.53 [a]	334.09	5.52
T3	9.09	64.75	55.66	0.48 [b]	322.27	5.79
Pooled SE	0.13	0.87	0.85	0.01	3.92	0.06

[1] Mean of six values

a, b Means with different superscripts within the column differ (P<0.05)

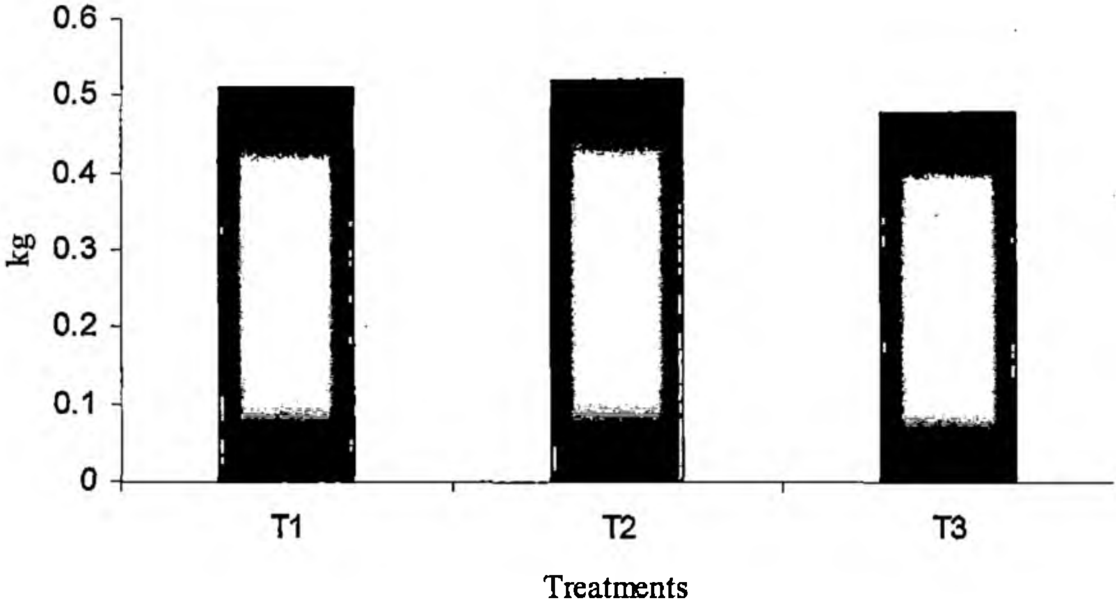

Fig.8. Average daily gain of pigs maintained on three experimental rations, experiment 2

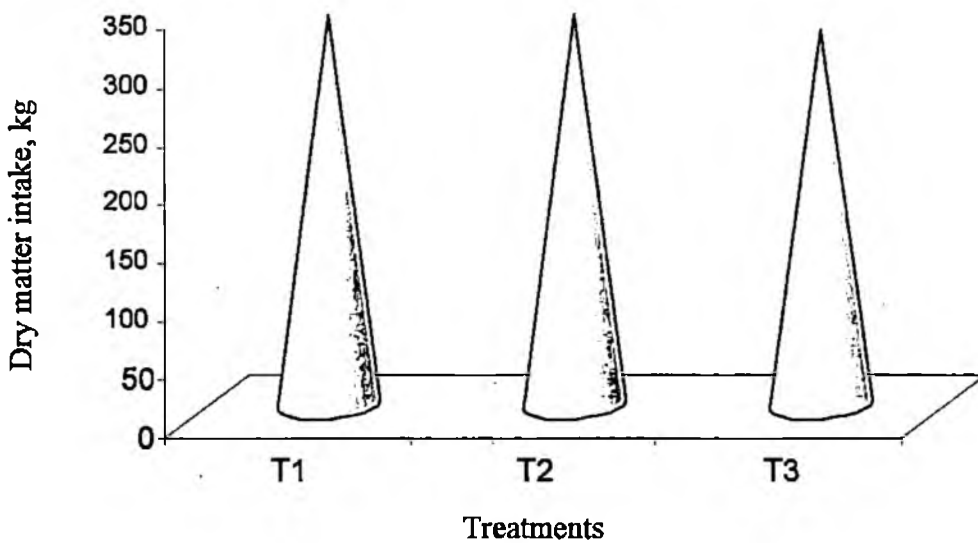

Fig.9. Total dry matter intake of pigs maintained on three experimental rations, experiment 2

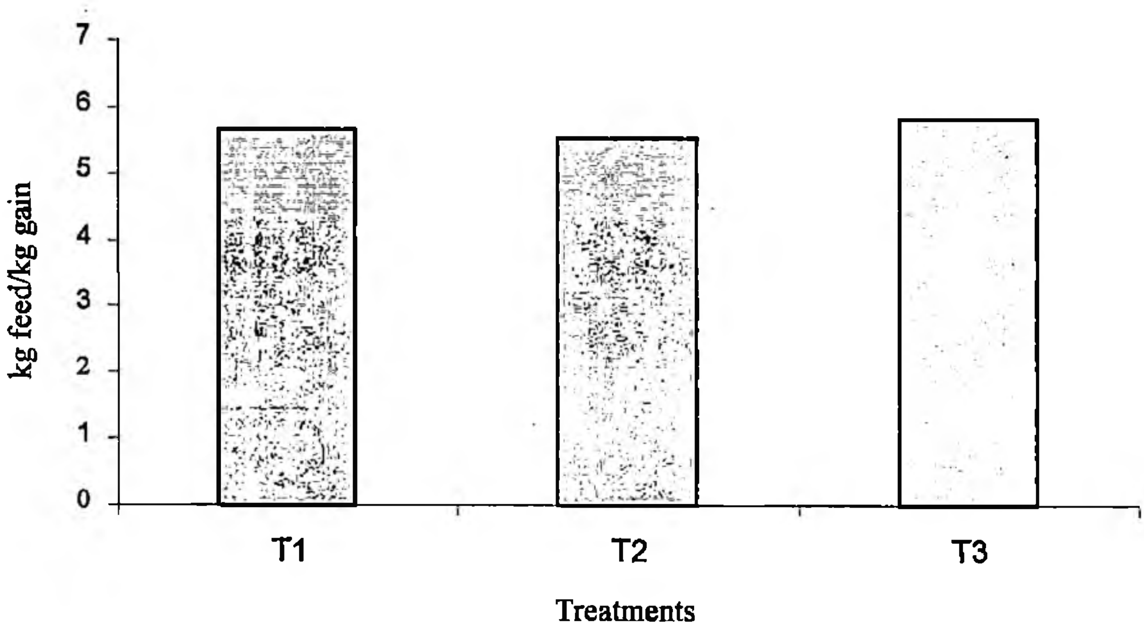

Fig.10. Feed efficiency of pigs maintained on three experimental rations, experiment 2

Table 20. Chemical composition of faeces of pigs maintained on three dietary treatments* of experiment 2

Item	Treatments		
	T1	T2	T3
Dry matter, %	30.09	28.68	30.64
Crude protein, %	19.64	19.75	20.90
Ether extract, %	8.79	10.67	10.22
Crude fibre, %	11.15	12.32	12.26
Total ash, %	20.47	17.69	17.32
Nitrogen free extract, %	39.95	39.57	39.30
Acid insoluble ash, %	5.54	7.78	7.89
Calcium, %	2.62	0.60	0.68
Phosphorus, %	2.03	0.92	0.97
Magnesium, %	0.77	0.72	0.76
Zinc, ppm	832.00	128.49	136.65
Copper, ppm	21.97	21.93	23.48
Manganese, ppm	26.16	25.98	27.74

* On DM basis

Table 21. Digestibility of nutrients[1] of three experimental rations of experiment 2, %

Treatments	Nutrients				
	Dry matter	Crude protein	Ether extract	Crude fibre	Nitrogen free extract
T1	86.11	83.63	52.81	55.49	92.22
T2	87.92	85.31	53.89	57.41	93.55
T3	87.95	84.97	53.89	55.94	93.56
Pooled SE	0.66	0.42	0.69	1.17	0.27

[1] Mean of six values

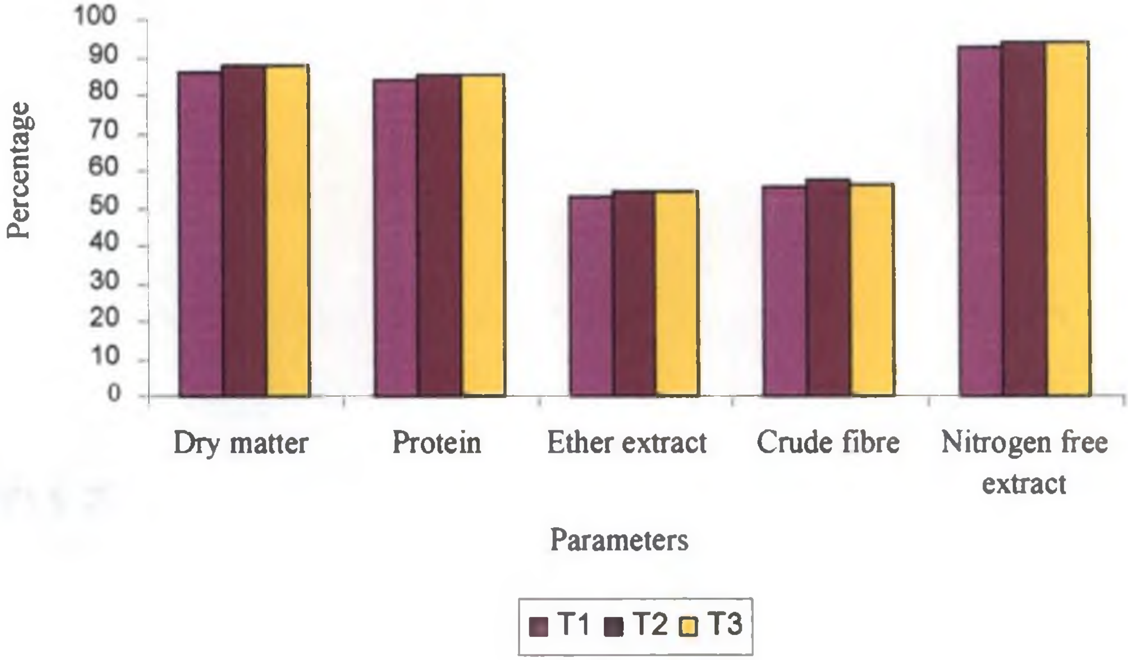

Fig.11. Digestibility of nutrients of three experimental rations, Experiment 2

Table 22. Availability of minerals[1] of three experimental rations of experiment 2, %

Treatments	Minerals					
	Ca	P	Mg	Zn	Mn	Cu
T1	52.03[a]	50.07[a]	68.15[a]	65.77[a]	72.13[a]	67.60
T2	64.68[b]	58.05[b]	64.20[b]	64.93[ab]	76.96[b]	70.68
T3	59.88[bc]	52.45[ab]	62.80[bc]	61.78[bc]	73.16[ac]	71.09
Pooled SE	1.59	1.37	0.81	0.78	0.62	1.17
P	0.00	0.04	0.01	0.08	0.00	0.44

[1] Mean of six values
a, b, c Means with different superscripts within each column differ

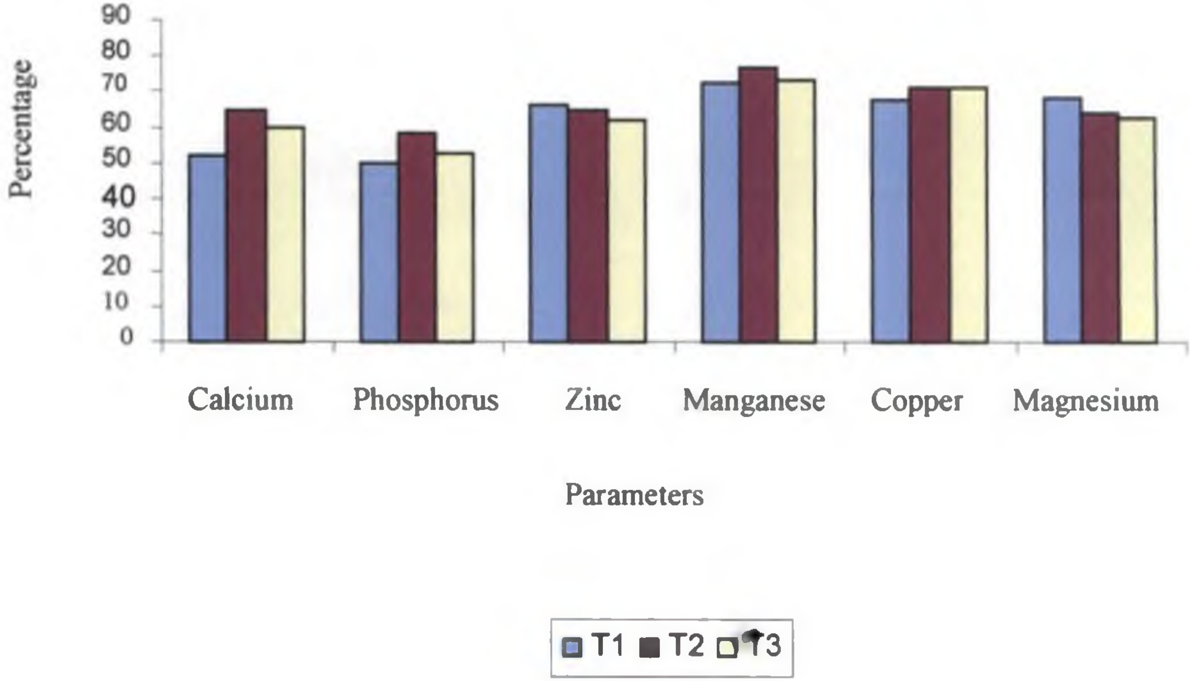

Fig.12. Availability of minerals of three experimental rations, experiment 2

Table 23. Plasma biochemical parameters of animals maintained on the experimental diets of experiment 2 [1]

Treatments	Calcium mg/dl	Phosphorus mg/dl	Magnesium mg/dl	Zinc ppm	Copper ppm	Manganese ppm	ALP U/L
T1	10.95	5.22	3.29	0.66	1.65	0.02	609.83
T2	10.56	5.58	3.79	0.68	1.69	0.02	552.67
T3	10.67	5.46	3.70	0.69	1.64	0.02	550.67
Pooled SE	0.21	0.08	0.12	0.04	0.04	0.00	12.32

[1] Mean of six values

Table 24. Carcass characteristics[1] of animals maintained on the three experimental rations of experiment 2

Treatments		T1	T2	T3	Pooled SE
Dressing Percent		73.76[a]	70.20[b]	70.16[bc]	0.55
Loin eye area (cm^2)		23.44[a]	23.38[a]	18.25[b]	0.15
Back fat thickness (cm)		3.40	3.41	3.39	0.08
Weight of Internal organs as percentage of curcass weight	Heart	0.40	0.40	0.40	0.01
	Lungs	1.51	1.48	1.50	0.06
	Liver	2.47	2.55	2.47	0.06
	Kidney	0.49	0.44	0.46	0.02
	Spleen	0.25	0.34	0.28	0.02
	stomach and intestine	13.41	15.37	13.46	0.43

[1] Mean of six values

a, b, c Means with different superscripts within each column differ

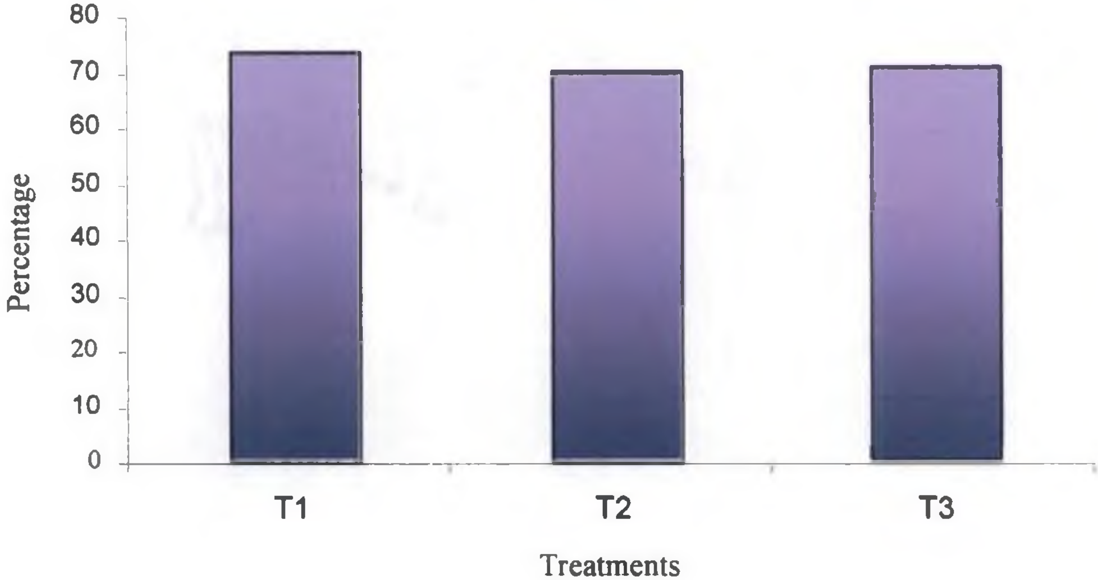

Fig.13. Dressing percentage of pigs maintained on three experimental rations, experiment 2

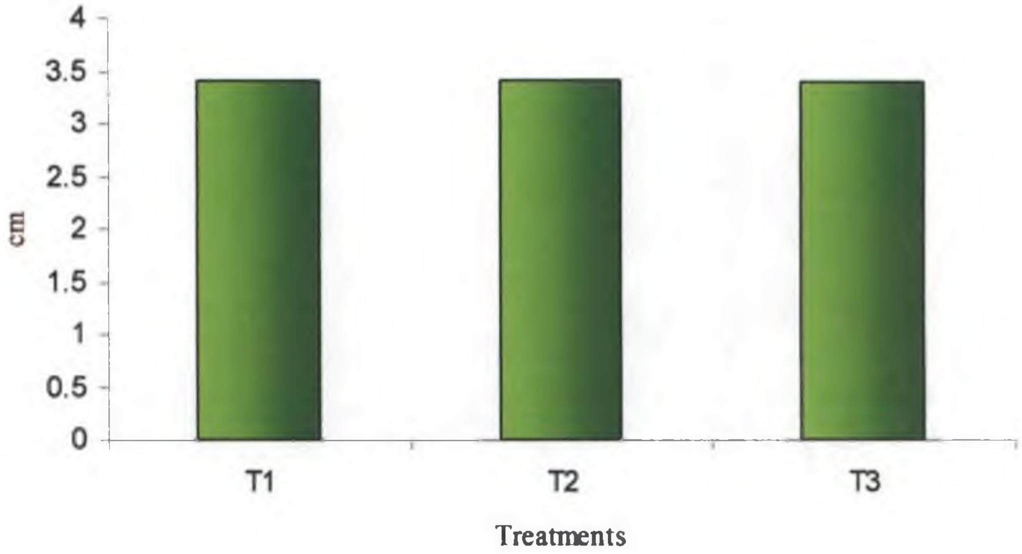

Fig.14. Back fat thickness of pigs maintained on three experimental rations, experiment 2

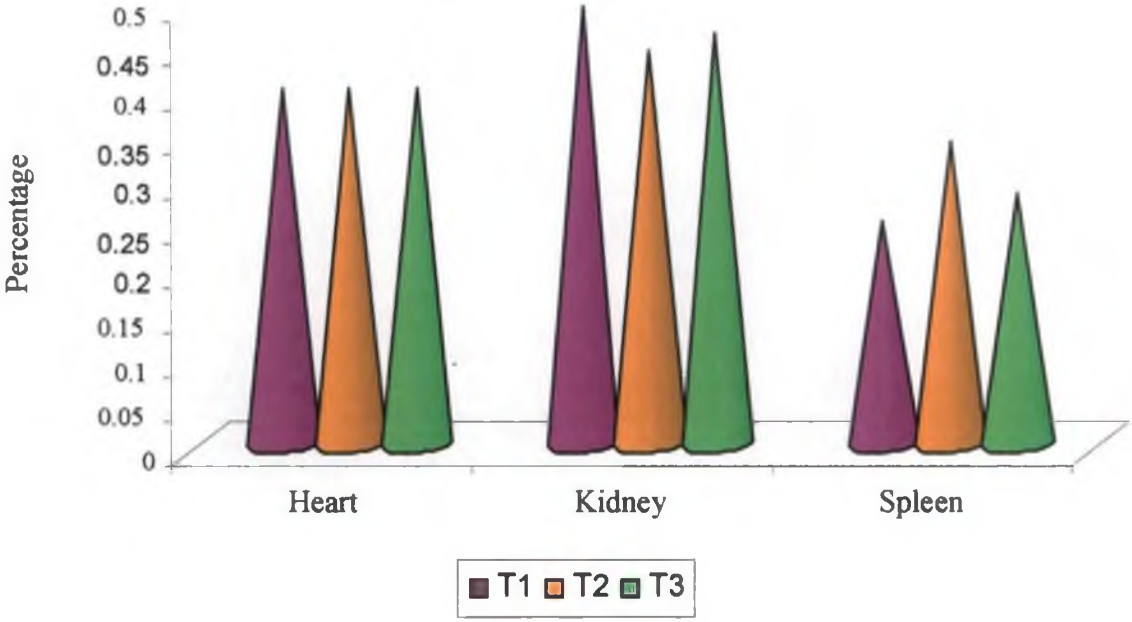

Fig.15. Weight of heart, kidney and spleen of the experimental animals as percentage of carcass weight, experiment 2

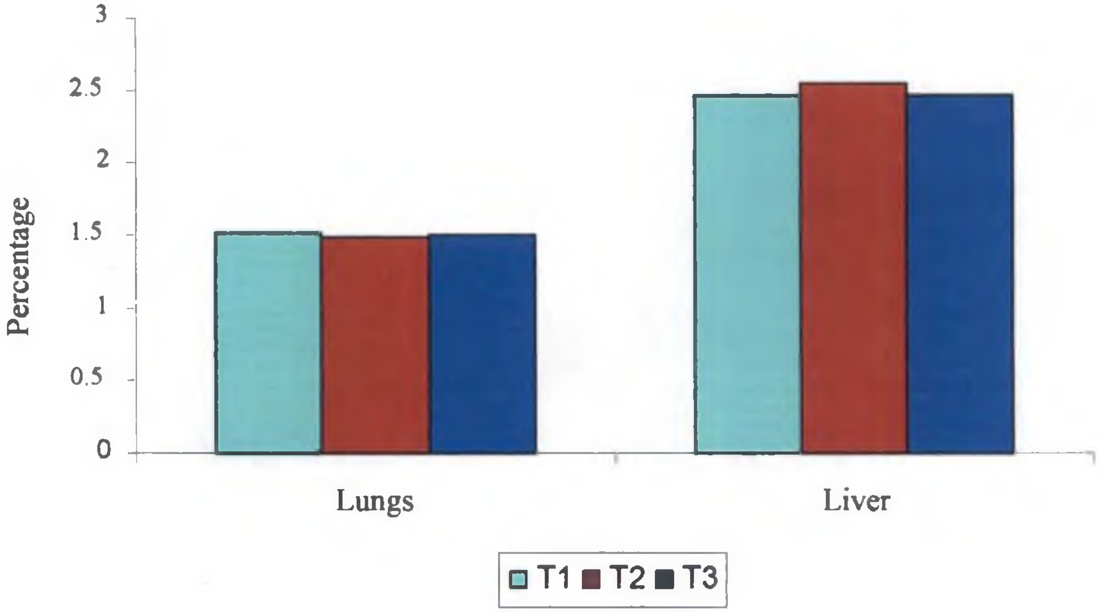

Fig.16. Weight of lungs and liver of experimental animals as percentage of carcass weight, experiment 2

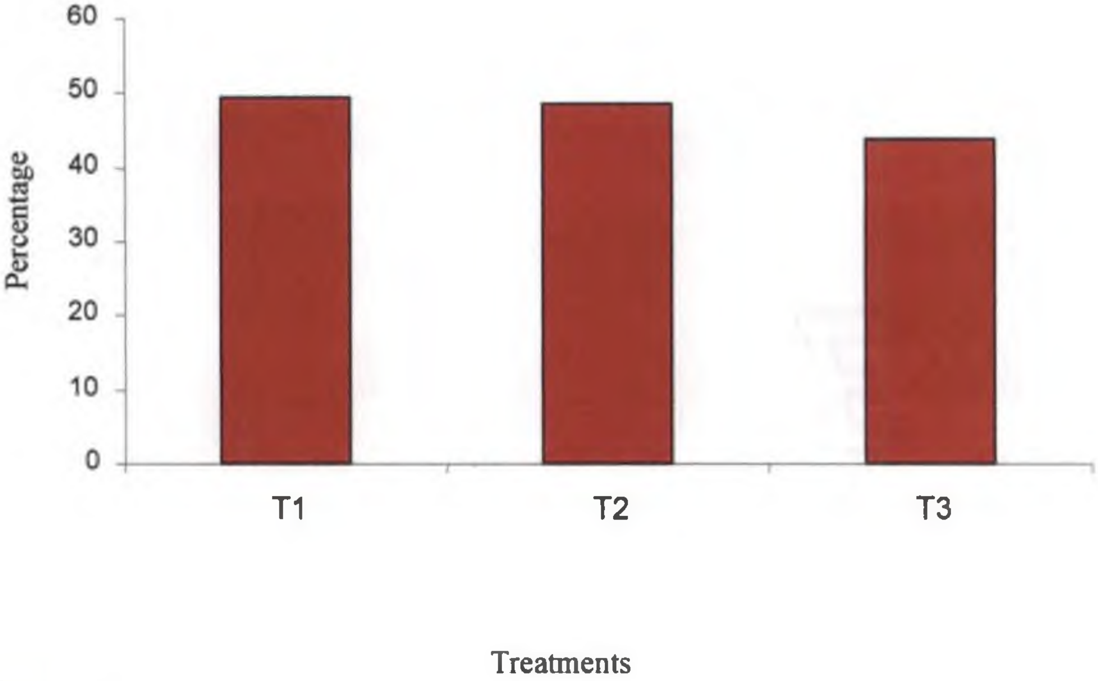

Fig. 17. Bone ash of pigs maintained on three experimental rations, experiment 2

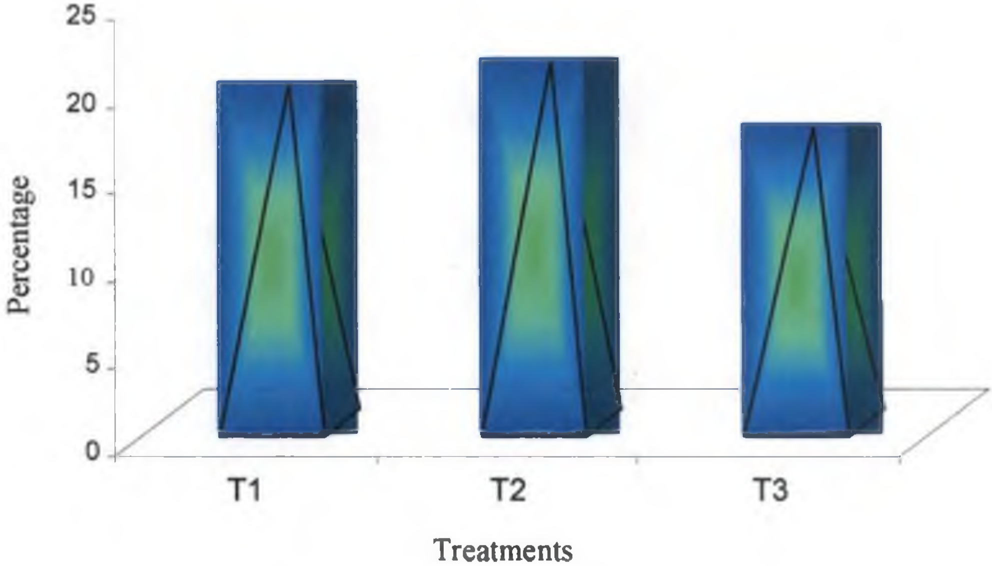

Fig.18. Calcium content of bones of pigs maintained on three dietary treatments experiment 2

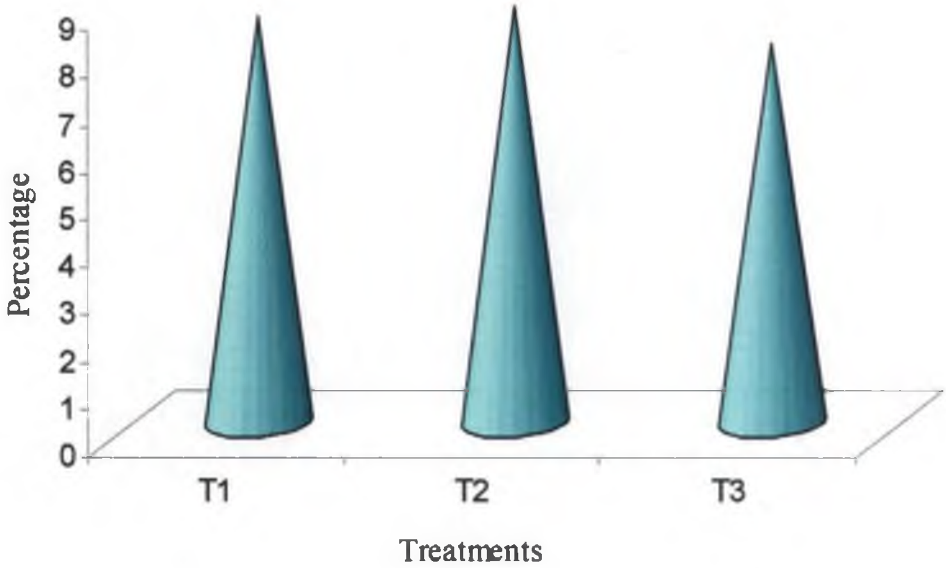

Fig.19. Phosphorus content of bone of pigs maintained on three dietary treatments, experiment 2

Table 25. Bone mineral [1] composition of animals maintained on the three experimental rations of experiment 2, %

Treatments	Bone ash	Calcium	Phosphorus
T1	49.62[a]	19.45	8.63
T2	48.66[a]	20.78	8.81
T3	43.69[b]	17.01	8.05
Pooled SE	0.89	0.83	1.58

[1] Mean of six values

a, b Means with different superscripts within each column differ (P<0.01)

Table 26. Cost of feed per kg body weight gain of pigs maintained on three dietary treatments in experiment 2

Item	Treatments		
	T1	T2	T3
Total body weight gain, kg	59.12	60.58	55.66
Total feed intake, kg	334.04	334.09	322.27
Cost /kg of starter ration, Rs.	11.71	10.97	10.89
Cost of finisher ration, Rs.	11.29	10.56	10.48
Total cost of feed, Rs.	3876.20	3630.62	3475.68
Cost of feed per kg body weight gain, Rs.	65.73	59.90	62.44

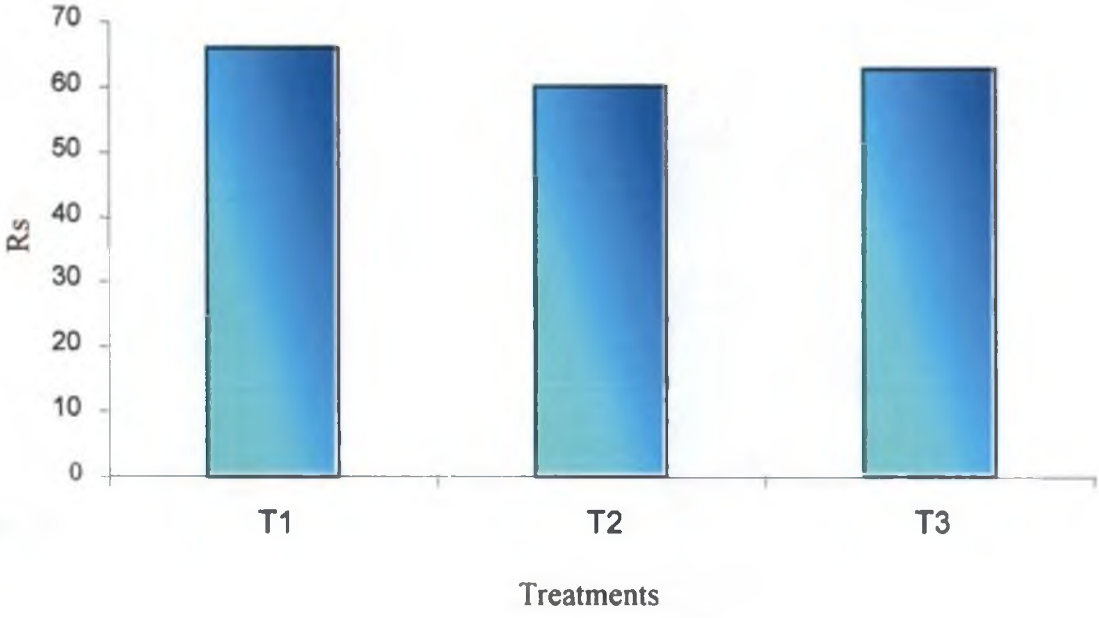

Fig.20. Feed cost per kg gain of pigs maintained on three experimental rations, experiment 2

Plate 4. Photograph of tenth rib of pig maintained on control ration T1 of experiment 2

Plate 5. Photograph of tenth rib of pig maintained on experimental ration T3 of experiment 2

Plate 6. X-ray of femur of pig maintained on control ration T1 of experiment 2

Plate 7. X-ray of femur of pig maintained on experimental ration T2 of experiment 2

Plate 8. X-ray of femur of pig maintained on experimental ration T3 of experiment 2

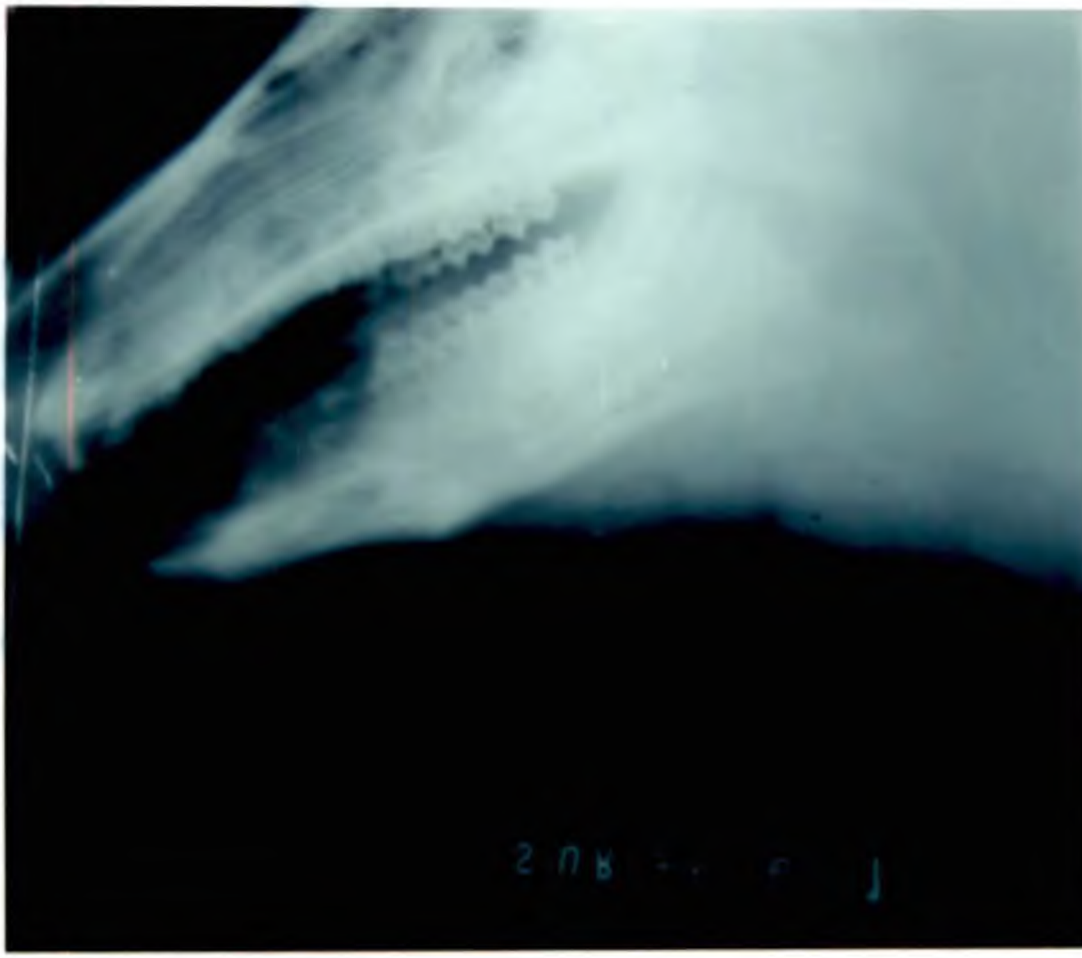

Plate 9. X-ray of mandible of pig maintained on control ration T1 of experiment 2

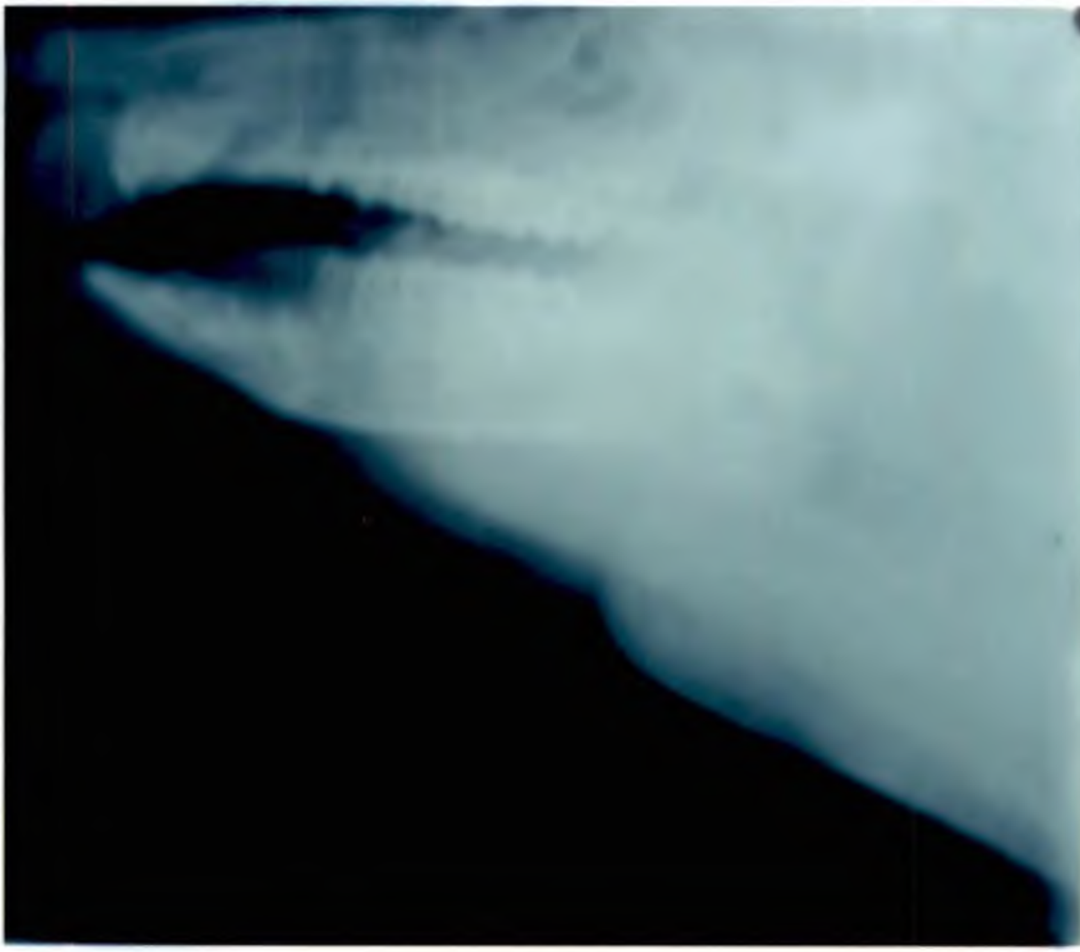

Plate 10. X-ray of mandible of pig maintained on experimental ration T2 of experiment 2

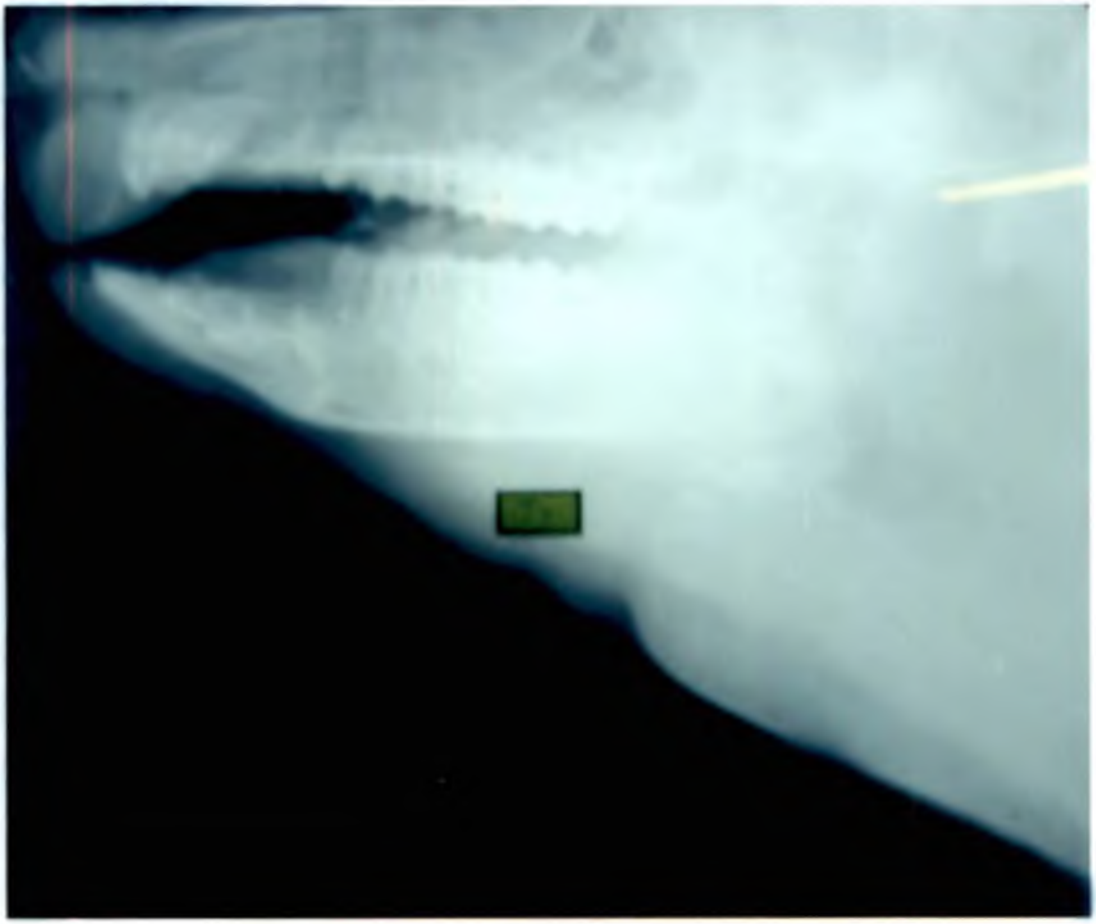

Plate 11. X-ray of mandible of pig maintained on experimental ration T3 of experiment 2

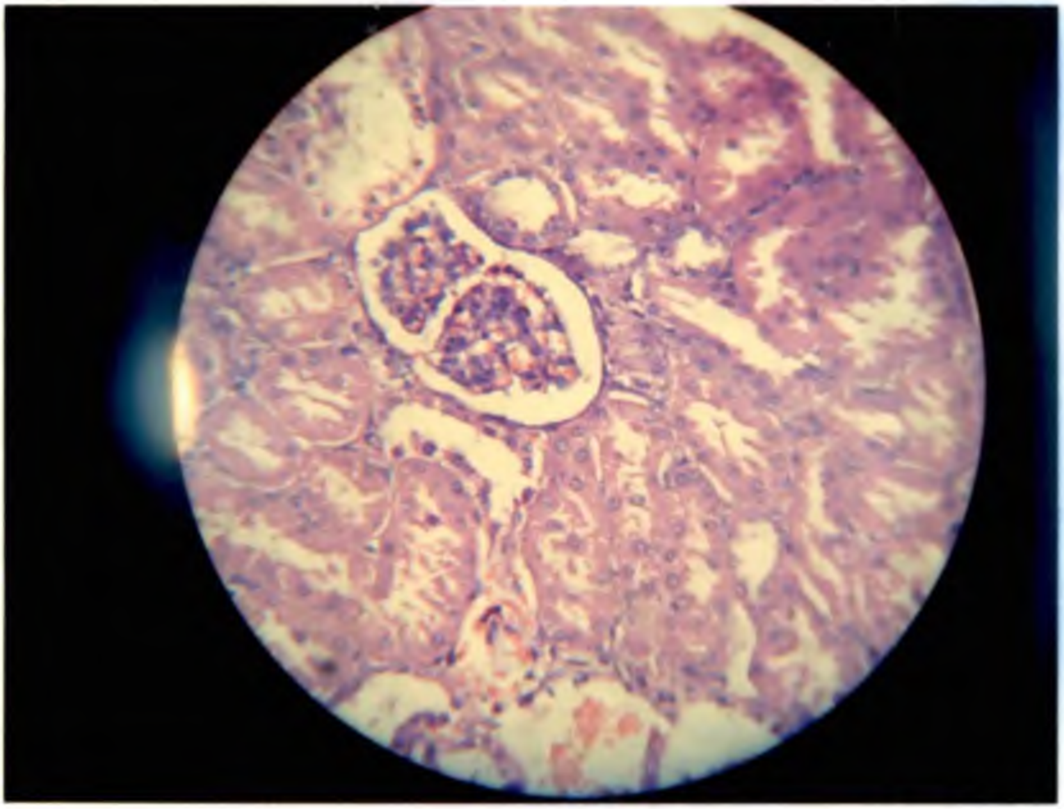

Plate 12. Histopathology kidney of pig maintained on control ration T1 of experiment 2

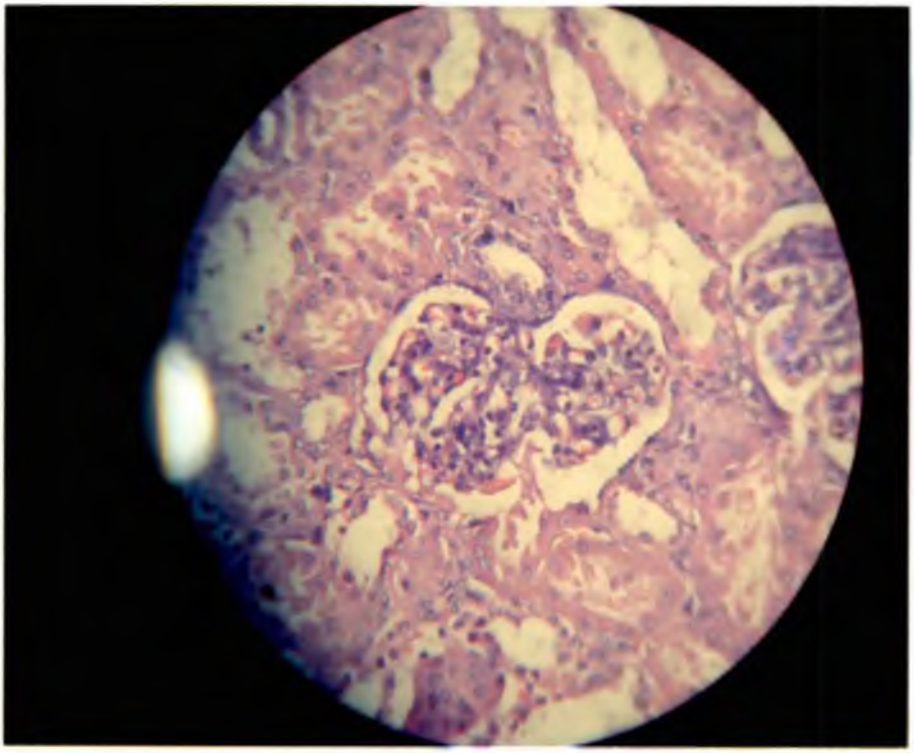

Plate 13. Histopathology kidney of pig maintained on experimental ration T2 of experiment 2

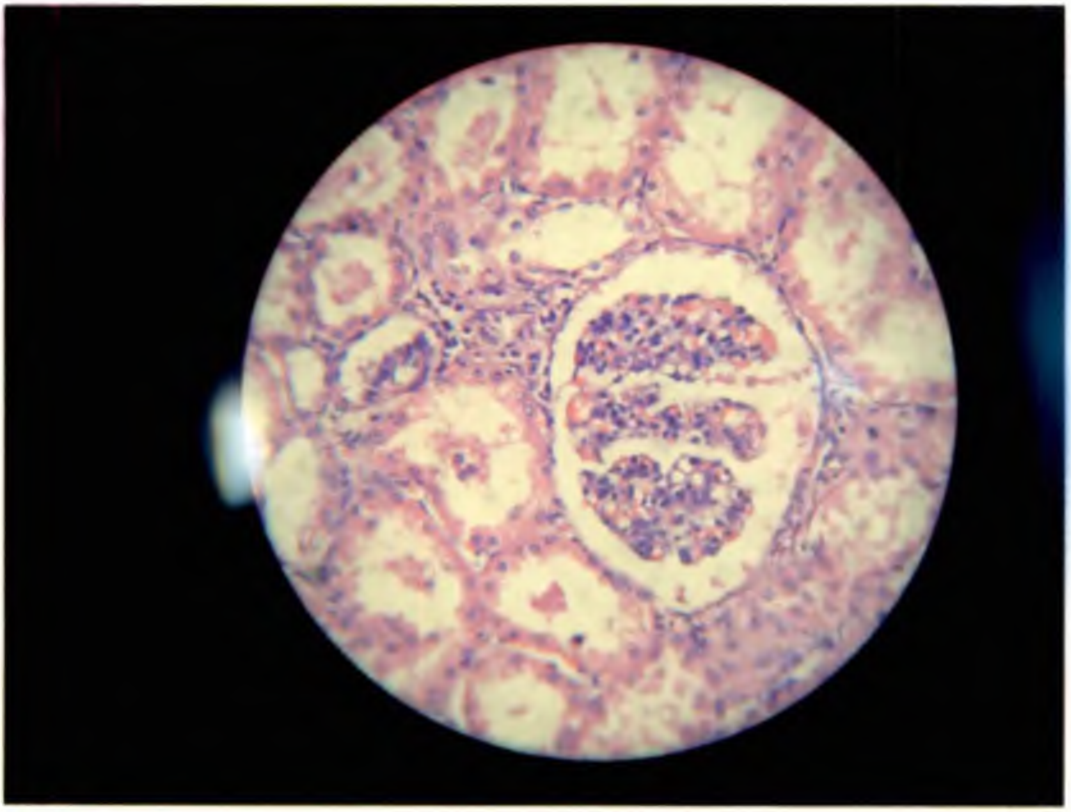

Plate 14. Histopathology of kidney of pig maintained on experimental ration T3 of experiment 2

Discussion

5. DISCUSSION

5.1 EXPERIMENT 1

5.1.1 Chemical Composition of Experimental Rations

Data on chemical composition of the grower and finisher swine rations are given in Tables 5 and 7, respectively. The percentage of moisture in grower rations varied between 12.80 and 15.15, ether extract between 1.29 and 1.81 and crude fibre between 1.2 and 1.99 per cent. The total ash and nitrogen free extract fraction varied between 4.85 and 6.1 and 72.51 and 73.41 per cent, respectively. The percentage of moisture in finisher rations varied between 10.96 and 12.74, ether extract and crude fibre between 1.03 and 1.48 and 2.65 and 3.35 per cent respectively. The total ash and nitrogen free extract varied between 5.24 and 6.98 and 72.52 and 73.8 per cent, respectively in the finisher rations.

The crude protein of the grower ration ranged from 18.02 to 18.57 per cent and that of finisher ration from 15.57 to 16.9 per cent. As per ICAR feeding standards (1985) ration for pigs weighing from 10 to 40 kg should contain 16 per cent CP and that of pigs weighing above 40 kg should contain 14 per cent CP. As per NRC (1998) the grower and finisher ration should contain 18 and 16 per cent CP respectively. The calculated ME value of the grower and finisher feeds were 3274 and 3294 kcals /kg, respectively which is in accordance with NRC (1998) recommendations of 3265 kcals/kg feed. According to ICAR (1985), the feed for growing and finishing swine should contain 68 per cent TDN and 3000 kcals of digestible energy /kg feed.

5.1.2 Mineral Composition

The Ca content of the eight grower rations (T1 to T8) was 0.67, 0.66, 0.64, 0.63, 1.04, 1.02, 1.02 and 1.07 per cent respectively. Similarly the P content of the feeds was 0.46, 0.46, 0.78, 0.76, 0.46, 0.45, 0.76 and 0.76 per cent respectively. The Ca content of finisher ration was 0.62, 0.64, 0.64, 0.62, 1.04, 1.08, 1.07 and 1.04 per cent respectively and P contents were 0.44, 0.45, 0.77, 0.78, 0.46, 0.46, 0.77 and 0.75 per cent, respectively.

As per ICAR (1985), the Ca and P requirement for pigs weighing from 10 to 40 kg are 0.6 and 0.5 per cent respectively and from 40 kg onwards, 0.5 and 0.4 per cent, respectively. The Ca and P requirements are 0.6 and 0.5 per cent for growers and 0.5 and 0.45 per cent for finishers respectively (NRC, 1998) which agrees well with that of ICAR 1985. Vipperman *et al.* (1974) suggested the dietary requirements to be 0.75 per cent Ca and 0.50 per cent P for growing pigs as evident from their excretion and retention data.

In the present study all the eight grower and finisher rations contained sufficient Ca to meet their requirements as per ICAR (1985) and NRC (1998). In rations T5, T6, T7 and T8 the Ca content was higher than the requirement values. Phosphorus content of experimental grower rations T1, T2, T5 and T6 are slightly below the requirement value while the P content of finisher rations were adequate to meet their demands. The different levels of Ca and P used in the present study were to study their effects and interaction, if any, with the utilization of other nutrients or with the growth performance of pigs.

Magnesium requirement of growers and finishers is 0.04 per cent (NRC 1998). The Mg content of the eight dietary treatments varied from 0.31 to 0.32 per cent respectively which are higher than the requirement. Zinc, Cu and Mn requirements for growers are 80, 5 and 3 mg respectively and 50, 4 and 2 mg /kg diet respectively for finishers (NRC 1998). The concentration of Zn, Cu and Mn varied between 335.99 to 353.19 ppm, 7.73 to 9.52 ppm and 13.53 to 15.24 ppm respectively for the eight grower rations in the present study and for finisher rations the concentration of Zn varied between 351.17 to 398.93 ppm, while that of Cu and Mn varied between 7.45 to 7.96 and 13.39 to 13.94 respectively. Zinc oxide was added to all the rations since higher Ca levels were used in the study which resulted in higher Zn concentration in all the experimental rations.

5.1.3 Body Weight and Average Daily Gain

Average final body weight of pigs belonging to eight treatments of the present study were 48.8, 50.31, 49.88, 49.5, 49.44, 49.88, 48.88 and 47.92 kg and the average daily gains were 388.00, 403.00, 399.50, 394.50, 393.25, 399.25, 384.00 and 375.00 g respectively. Data on average body weight of the animals at fortnightly interval and ADG are given in Tables 9 and 10 and depicted in Fig.1 and 2, respectively. On perusal of the data there were no significant differences (P >0.05) in the body weight of pigs fed low Ca (T1, T2, T3 and T4) and high Ca (T5, T6, T7 and T8) containing rations. No difference (P >0.05) was observed in the body weight of pigs fed low P (T1,T2, T5 and T6) and high P (T3, T4, T7 and T8) rations.

Kornegay *et al.* (1981), Nimmo *et al.* (1981) and Crenshaw (1986) in their experiments with different levels of Ca and P opined that ADG in pigs was not affected by higher levels of Ca and P (0.6 to 1.2 per cent Ca and 0.6 to 0.8 per cent P) which concurs with the results obtained during the present study. Reinhart and Mahan (1986) suggested that high Ca: P ratios can adversely affect swine performance and when low P is provided Ca: P ratios above 1.3:1 resulted in decreased growth whereas when high dietary P levels were provided negative effects were exhibited when the Ca: P ratio exceeded 2.0:1. Cera and Mahan (1988) also observed maximum gains at 0.65:0.5 per cent and 0.52:0.4 per cent of Ca and P respectively, during the grower and finisher periods.

Addition of phytase did not influence the body weight of pigs in the present experiment. Kemme *et al.* (1997), Radcliffe *et al.* (1998), Moreira *et al.* (2003), Park *et al.* (2003) and Cervantes *et al.* (2004) also observed that ADG was not significantly affected by the addition of phytase in the diet of pigs. Akyurek *et al.* (2005) observed that phytase supplementation did not affect body weight gain in broilers.

Enhanced body weight gain in pigs was reported by Lei *et al.* (1993), Adeola *et al.* (1995) and Biehl *et al.* (1996) as a result of phytase supplementation. Similarly

Cromwell *et al.* (1993) found that pigs restored body weight on addition of phytase to low P diets. Harper and Kornegay (1996), Harper *et al.* (1997), Liu *et al.* (1997), Murry *et al.* (1997) and O'Quinn *et al.* (1997) also observed linear increase in growth performance in pigs when graded levels of phytase was supplemented to low P diets. Matsui *et al.* (2000), Landblom *et al.* (2002), Gentile *et al.* (2003), Augspurger *et al.* (2004a), Omogbenigun *et al.* (2004), Shelton *et al.* (2004), Jendza *et al.* (2005), Kies *et al.* (2005), Brana *et. al* (2006) and Veum *et al.* (2006) also recorded increased ADG consequent to phytase supplementation of pig diets while Pillai *et al.* (2006) observed that increasing iP resulted in a linear increase in weight gain and adding *E. coli* phytase resulted in a linear and quadratic increase in weight gain in broilers.

In contrast with the above findings Qian *et al.* (1996) reported that the activity of phytase was decreased as the Ca:tP ratio became wider as evidenced by a decreased ADG. Williams *et al.* (2005) on the contrary observed that dietary phytase decreased ADG in pigs fed diets containing 1000 or 2000 ppm of Zn.

5.1.4 Dry Matter Intake

Data on DMI of pigs maintained under the eight dietary treatments T1, T2, T3, T4, T5, T6, T7 and T8 are presented in Table 10 and are graphically represented in Fig.3. Dry matter intake recorded for the eight dietary treatments were 179.22, 190.310, 186.69, 189.69, 190.16, 187.39, 184.96 and 181.72 kg, respectively. On statistical analysis of the data there were no significant ($P > 0.05$) effects of dietary Ca, P and phytase levels on DM intake. However significant interactions ($P<0.05$) were observed between the Ca level and P level of the experimental rations and also between ($P<0.01$) Ca level and phytase level of the experimental ration on DM intake of the animals. Animals fed ration containing high Ca and low P (T5), showed significantly higher DM intake than those fed high Ca and high P (T7). It was also observed that the animals fed low Ca ration without phytase (T1) exhibited significantly low DM intake than those fed rations containing low Ca ration with phytase (T2). It was also observed that there was no three way interaction between the dietary Ca, P and phytase levels on DM intake of pigs.

Pond *et al.* (1978) observed higher feed intake with high Ca diets and Nimmo *et al.* (1981) observed a lower daily feed intake for gilts fed low Ca:P diet (0.65 per cent Ca and 0.50 per cent P) in concurrence with the findings of the present study. Lei *et al.* (1993), Liu *et al.* (1997), Kies *et al.* (2006) and Guy *et al.* (2008) indicated that supplemental phytase significantly enhanced feed intake in pigs. Dilger *et al.* (2004) also observed that a new microbial phytase (Phyzyme XP) increased feed intake in broiler chicks which agrees well with that of the present study.

On the contrary, Kornegay *et al.* (1981a) found no overall difference in daily feed intake between boars fed diets containing Ca and P at 100, 125 and 150 per cent of NRC recommendations and with two protein levels. They also did not observe any interactions between the dietary Ca, P and protein content in the diet. O'Quinn *et al.* (1997) also observed that decreasing dietary P content by 25 per cent of NRC recommendations did not affect feed intake during growth period.

Han *et al.* (1997), Moreira *et al.* (2003), Park *et al.* (2003), Shelton *et al.* (2004), Jendza *et al.* (2005), Kies *et al.* (2005) and Williams *et al.* (2005) opined that phytase supplementation did not affect average daily feed intake inpigs. Phytase supplementation did not affect feed intake in broiler chicks as reported by Onyango *et al.* (2004) and Akyurek *et al.* (2005) and in rabbits by Xian *et al.* (2004).

5.1.5 Feed Conversion Efficiency

The feed conversion efficiency of pigs belonging to the eight experimental treatment groups were 5.25, 5.38, 5.32, 5.49, 5.50, 5.33, 5.48 and 5.51 respectively (Table 10 and Fig.4). There was no significant differences ($P > 0.05$) for the feed conversion efficiency between rations containing low as well as high Ca and P and also between the rations with (750 units/kg) and without phytase supplementation.

Kornegay *et al.* (1981), Nimmo *et al.* (1981), Crenshaw (1986) and Cera and Mahan (1988) obtained similar results as in the present study, where they found no effect on feed : gain ratio when fed diets containing varying Ca and P levels. Reinhert and Mahan (1986) opined that pigs fed low P diets displayed lower feed efficiency after the Ca:P ratio exceeded 1.3:1, whereas negative effects of feeding high dietary P levels were exhibited only when the Ca:P ratio exceeded 2.0:1.

No effect of phytase supplementation on feed conversion was reported in growing pigs by Kemme *et al.* (1997), O'Quinn *et al.* (1997) Moreira *et al.* (2003) Cervantes *et al.* (2004) and Martinez *et al.* (2004) which is in concurrence with that of present study.

Boling *et al.* (2001) observed that phytase addition had no significant effect on PER values for any of the ingredients evaluated, except for casein in chicks. Hariharan (2003) also observed no significant increase in feed efficiency and protein efficiency in broiler chicks fed diets with citric acid or phytase. These results are in agreement with the results of the present study though the present study was in pigs.

Cromwell *et al.* (1993), Lei et *al.* (1993), Adeola *et al.* (1995), Biehl *et al.* (1996) and Qian *et al.* (1996) on the other hand documented improved feed efficiency with supplementation of phytase in pigs. Han *et al.* (1997) obtained similar feed efficiency of pigs when fed diets supplemented with microbial phytase, cereal phytase or iP indicating that supplementation of phytase can completely replace iP in the diet of pigs. Liu *et al.* (1997) also observed that phytase supplementation of the low P diet increased gain:feed ratio linearly with increasing levels of microbial phytase in pigs. Murry *et al.* (1997) also observed that gain: feed ratio was linearly increased with increasing levels of phytase in pigs fed low P diets. However, the gain:feed ratio decreased in pigs fed the adequate P diets supplemented with phytase while Radcliffe *et al.* (1998) observed that addition of citric acid and phytase to corn-soyabean meal based diet low in Ca and P improved feed efficiency. Sands *et al.* (2001) observed better feed efficiency in pigs fed phytase supplemented HAP corn diet and Park *et al.* (2003) noticed better feed efficiency by the addition of a solid state fermented phytase complex to low aP, corn- soyabean meal diet in pigs.

Williams *et al.* (2005) found that gain:feed ratio was linearly increased by Zn and phytase. Brana *et al.* (2006) also observed a linear increase in gain:feed ratio in nursery and grower pigs subsequent to dietary addition of phytase whereas in finisher pigs phytase supplementation increased gain:feed ratio quadratically.

5.1.6 Digestibility of Nutrients

5.1.6.1 Dry Matter

Chemical composition of faeces of pigs fed with different experimental diets is shown in Table 11. Data on apparent per cent digestibility of nutrients is presented in Table 12 and is graphically represented in Fig.5. The percentage DM digestibility of the eight experimental rations T1, T2, T3, T4, T5, T6, T7 and T8 were 86.95, 83.72, 84.16, 84.85, 83.88, 85.29, 80.59 and 83.61 respectively.

. The statistical analysis of the data revealed that DM digestibility was significantly affected by dietary Ca ($P<0.05$) and P ($P<0.01$) levels. Ration with high Ca and high P (T7) registered lower ($P<0.01$) DM digestibility than the ration with low Ca and low P (T1). It was further observed that dietary phytase had no effect on DM digestibility.

On two way analysis of the data, significant interaction ($P<0.01$) between dietary Ca and phytase level on DM digestibility was observed. Ration containing high Ca without phytase supplementation (T5) showed significantly ($P<0.01$) low DM digestibility than the other groups. There was also interaction ($P<0.05$) on DM digestibility between the P and phytase levels of the ration. Ration containing high P and low Ca without phytase (T3) was showing significantly low ($P<0.05$) DM digestibility.

Interaction between dietary Ca, P and phytase on DM digestibility was found nonsignificant ($P >0.05$).

In agreement to the results obtained in the present study, Mroz *et al.* (1994), Johnston *et al.* (2004), Omogbenigun *et al.* (2004)), Kies *et al.* (2005), Kies *et al.*

(2006) and Viswanathan *et al.* (2007) observed higher DM digestibility in pigs as a result of phytase supplementation.

Akyurek *et al.* (2005) observed significant improvement in ileal DM digestibility when phytase was added to low aP containing diets while Juanpere *et al.* (2005) reported enhanced DM digestibility only on corn diets.

Harper *et al.* (1997) on the other hand, observed no significant effect of dietary P or phytase level on DM digestibility in the grower phase of swine. No effect on apparent DM digestibility was recorded by O'Quinn *et al.* (1997), Park *et al.* (2003) and Jendza *et al.* (2005) in pigs, while Dilger *et al.* (2004) and Cowieson and Adeola (2005) reported in broilers and Anaya *et al.* (2008) in dogs.

On contrary, Kemme *et al.* (1997) observed reduced DM digestibility in piglets when phytase was supplemented to corn –soyabean meal based diets.

In the present study, a significant interaction between P level and phytase was observed for DM digestibility. However Murry *et al.* (1997) on the contrary did not observe any interaction between P level and phytase on DM digestibility. Sands *et al.* (2001) obtained an interaction between corn and phytase on DM digestibility when diets containing HAP corn or normal corn were supplemented with phytase.

5.1.6.2 Crude Protein

Data on percentage digestibility of CP obtained during the present study is represented in Table 12 and graphically represented in Fig.5.

The CP digestibility values for the eight dietary treatments obtained during the present study were 81.18, 81.85, 79.00, 79.27, 78.93, 80.73, 80.62 and 78.36 per cent, respectively. On statistical analysis of the data it was observed that there was no significant difference ($P > 0.05$) or interactions between the dietary treatments with regard to protein digestibility in the present investigation.

O'Quinn *et al.* (1997) observed in growing-finishing pigs fed sorghum-soyabean meal based diets that decreasing dietary P did not affect apparent digestibility of nitrogen and their results agrees with the result of the present investigation. Traylor *et al.* (2001) and Cervantes *et al.* (2004) also observed that ileal digestibility of N was not affected by phytase supplementation in swine. No effect of phytase on the apparent digestibility of CP was also reported by Liao *et al.* (2005). They concluded that addition of phytase to diets formulated with commonly used feed ingredients did not improve the apparent ileal digestibilities of CP and amino acids in weanling pigs and that the amino acid response factor to microbial phytase supplementation depends on diet composition. Jendza *et al.* (2005) reported that adding phytase to the basal diet without supplemental iP had no effect on apparent digestibility of N. Akyurek *et al.* (2005) obtained similar CP digestibility for diets with low or adequate aP in broiler diets.

In contrast to the results of the present study Mroz *et al.* (1994) observed significant enhancement of apparent digestibility of CP in pigs fed corn-tapioca-soyabean meal diet supplemented with microbial phytase. Murry *et al.* (1997) and Omogbenigun *et al.* (2004) also observed that adding phytase increased CP digestibility linearly in the low P diets and CP digestibility linearly and quadratically in the P adequate diets. Similarly Johnston *et al.* (2004) and Kies *et al.* (2005) observed increased ileal digestibility of various amino acids and N in pigs supplemented with phytase.

Ravindran *et al.* (1999) observed that addition of microbial phytase to broiler diets improved the digestibility of protein and amino acids in all feed stuffs tested but the magnitude of response varied depending on the feed stuff and the amino acid considered. Onyango *et al.* (2005) also observed that newly evolved *E. coli* phytase improved retention of N and amino acids such as arginine, histidine, threonine, tryptophan, valine, aspartate and proline in poultry.

5.1.6.3 Ether Extract

The percentage EE digestibility values for the eight experimental rations of the present investigation are given in Table 12 and are depicted in Fig.5. Percentage

EE digestibility values of the eight experimental rations T1, T2, T3, T4, T5, T6, T7 and T8 were 33, 28.6, 32.50, 26.37, 30.13, 37.33, 21.90 and 21.37, respectively.

Statistical analysis of the data revealed that level of P in the diet was significantly ($P<0.01$) affecting the EE digestibility. Rations containing high P and high Ca (T7 and T8) were showing significantly ($P<0.01$) lower EE digestibility than the rest of the ration. There was no significant ($P >0.05$) effect of level of Ca and phytase in the ration on EE digestibility.

On two way analysis dietary Ca x P interaction was found significant ($P<0.01$) for EE digestibility in pigs. Ration containing high Ca and high P (T7 and T8) was showing significantly ($P<0.01$) low EE digestibility than the rations containing low Ca low P (T1), low Ca and high P (T3) and also with ration containing high Ca and low P (T5). There was also an interaction between dietary Ca and phytase ($P<0.05$) on EE digestibility. The ration containing low level of Ca without phytase (T1) was found to have significantly ($P<0.05$) higher EE digestibility than T2 or T4, T5 or T7 and T6 or T8.

There was no significant ($P >0.05$) interaction between dietary Ca, P and phytase levels on EE digestibility.

Kies *et al.* (2005) observed that phytase supplementation increased apparent digestibility of fat by 1.2 per cent. In the present study EE digestibility was increased when phytase was supplemented to high Ca diet. Similarly, Viswanathan *et al.* (2007) observed higher EE digestibility for citric acid and citric acid plus phytase supplemented ration than that of control ration.

Significant improvement in ileal digestibility of EE was reported by Akyurek *et al.* (2005) when phytase was supplemented to corn based diets of broiler birds fed diets containing 0.45 per cent of aP.

5.1.6.4 Crude Fibre

Data on digestibility of crude fibre (CF) of the eight experimental rations are given in Table12 and are depicted in Fig.5. Crude fibre digestibility values of the eight rations were 45.07, 42.13, 42.95, 51.00, 43.80, 45.43, 43.17 and 46.4 respectively. On statistical analysis of the data it was observed that level of phytase in the diet significantly ($P<0.05$) affected CF digestibility of the rations. Rations containing low level of Ca along with high P and phytase (T4) and also ration containing high levels of Ca, P and supplemented with phytase (T8) showed significantly ($P<0.05$) higher CF digestibility than the other raions.

On two way analysis of the data it was observed that there was significant interaction ($P<0.05$) between dietary P and phytase on CF digestibility. Rations containing high P (0.6 per cent) and supplemental phytase was showing significantly ($P<0.05$) higher CF digestibility than those rations with low Ca with or without phytase (T1, T5, T2, T6) and rations containing high Ca without phytase (T3 and T7). There was no three way interaction between dietary levels of Ca, P and phytase.

Viswanathan *et al.* (2007) observed that the digestibility coefficient of crude fibre was significantly higher for citric acid and citric acid plus phytase supplemented groups than that of control which agrees well with the result of the present study.

5.1.6.5 Nitrogen Free Extract

Data on NFE digestibility pertaining to the present study is shown in Table 12 and is depicted in Fig.5. The percentage NFE digestibilities of the eight dietary treatments were 93.38, 92.65, 91.32, 92.2, 91.64, 92.57, 92.44 and 91.75, respectively. The data showed no significant ($P>0.05$) difference between the treatment groups for NFE digestibility.

Viswanathan *et al.* (2007) observed that supplementation of citric acid and phytase supplemented diets showed improved NFE digestibility whereas in the present study there was no effect of phytase supplementation on NFE digestibility.

5.1.7 Availability of Minerals

Data on per cent availability of minerals are presented in Table 13 and are graphically represented in Fig.6.

5.1.7.1 Calcium Availability

In the present study the percentage Ca availability of the eight dietary rations T1 to T8 were 33.70, 36.30, 36.03, 29.77, 29.24, 50.45, 32.25 and 41.08, respectively.

The statistical analysis of data showed that there was significant ($P<0.01$) difference on Ca availability of the different rations due to supplementation of phytase. All the phytase supplemented groups had better Ca availability except the ration T4 containing 0.6 per cent Ca and 0.6 per cent P.

On two way analysis Ca x phytase interaction was found significant ($P<0.01$). Rations containing low levels of Ca with or without phytase (T1 and T3) and ration containing high level of Ca without phytase (T5 and T7) gave similar results whereas ration containing high level Ca with phytase (T6 and T8) showed significantly ($P<0.01$) better Ca availability than other three rations. There was also dietary P x phytase interaction ($P<0.05$) for Ca availability. Rations containing low P with phytase (T2 and T6) was found significantly better than rations containing higher P level with (T4 and T8) or without phytase supplementation (T3 and T7) as well as ration containing low P without supplemented phytase (T1 and T5).

This is in agreement with Lei *et al.* (1993) who observed that supplemental phytase significantly enhanced retention of Ca and P in weanling pigs. Mroz *et al.* (1994) and Adeola *et al.* (1995) also observed a significant enhancement of apparent digestibility of Ca as a result of phytase supplementation. Kemme *et al.* (1997) observed that digestibilities of Ca and P were not different between the groups of piglets fed either *ad libitum* or rationed amounts of feed. They also observed that, irrespective of piglet weight, phytase supplementation enhanced the digestibilities of Ca and P by 4.6 and 13.6 percentage units respectively. Liu *et al.* (1997), Murry *et al.* (1997) and O'Quinn *et al.* (1997) also noted that supplemental phytase increased Ca absorption in pigs. Liu *et al.* (1998) also found that lowering the Ca : tP ratio, linearly decreased Ca intake and apparent Ca absorption but quadratically increased the apparent percentage of Ca digestibility with phytase supplementation. Sauer *et al.* (2003) and Johnston *et al.* (2004) found that supplementation of phytase to the barley-canola meal and the barley-soyabean meal diets in growing pigs increased digestibility of Ca. Increased Ca and P digestibility in broilers was reported by Akyurek *et al.* (2005) with phytase supplementation at 0.5 g/kg to broiler diets and Veum *et al.* (2006) also noticed greater apparent absorption of Ca, consequent to phytase supplementation in pigs.

Liu *et al.* (2000) on the other hand found that lowering the dietary Ca:tP ratio in the diets containing phytase linearly increased the apparent absorption of P in the small intestine but Ca absorption was not affected.

Xian *et al.* (2004) observed in rabbits that the dietary phytase remarkably reduced excretion of Ca.

Murry (1995) noticed a P x phytase interaction on Ca digestibility. He could observe an increased Ca digestibility for those pigs fed low P diets with microbial phytase and in the present study also P x phytse interaction and Ca x phytase interaction was found significant for calcium availability.

5.1.7.2 Phosphorus Availability

Data on percentage availability of P revealed that the eight experimental rations T1, T2, T3, T4, T5, T6, T7 and T8 had the following per cent availability of P as 38.93, 44.47, 50.18, 48.80, 31.71, 47.07, 39.33 and 43.31. On statistical analysis it was observed that levels of Ca, P or phytase did not have any significant ($P>0.05$) effect on availability of P. No interaction was noted between Ca, P or phytase levels of the diet. However animals belonging to the group fed with the dietary treatment T5 (containing high level Ca and low level P without supplemental phytase) was found to have a lower P availability than that of T3 and T4 (containing low levels of Ca and high levels of P with and without phytase).

O'Quinn *et al.* (1997) indicated an increase in apparent digestibility of P on decreasing dietary P in the diet while Lei *et al.* (1993), Mroz *et al.* (1994) Adeola *et* *al.* (1995) and Han *et al.* (1997) observed that supplemental phytase significantly enhanced retention of P, apparent digestibility of tP and apparent P balance in weanling pigs. Similarly Harper and Kornegay (1996) observed enhanced P digestibility with phytase supplementation of low P diet in growing finishing pigs. They noted that P digestibility coefficients were 28.7, 32.6 and 41.3 per cent for diets containing 0, 167, 333 and 500 PU/ kg feed.

Kemme *et al.* (1997) also observed that phytase supplementation enhanced the digestibility of P. Harper *et al.* (1997) observed that reducing P level in the diet resulted in a reduction in P digestibility in the grower and finisher phase, but addition of phytase to the low P diet resulted in a linear improvement in P digestibility in the grower and finisher phases and for the pooled phases. Murry *et al.* (1997) and Liu *et al.* (1997) also noticed that apparent P absorption was increased linearly with graded levels of phytase.

Han *et al.* (1997) observed that the retention of total ingested P increased by 14 and 19 per cent respectively in pigs supplemented with microbial phytase and iP. Liu *et al.* (1998), Liu *et al.* (2000), Matsui *et al.* (2000), Gentile *et al.* (2003), Park *et al.* (2003) and Sauer *et al.* (2003) suggested that phytase supplementation improved the apparent digestibility of P while Johnston *et al.* (2004) in their study in pigs found that reducing the Ca and P in the diet increased the ileal digestibility of Ca and P and phytase supplementation increased the ileal digestibility of Ca and P further.

Subsequently Omogbenigun *et al.* (2004), Jendza *et al.* (2005), Brana *et al.* (2006), Nyachoti *et al.* (2006) and Veum *et al.* (2006) also opined that phytase supplementation improved P digestibility and availability in pigs. Akyurek *et al.* (2005) also observed that phytase supplementation at 0.5 g/kg to broiler diets significantly increased P retention.

Xian *et al.* (2004) also observed in rabbits that the dietary phytase remarkably reduced the excretion of total P.

O'Quinn *et al.* (1997) could notice that supplemented phytase at increasing levels reduced fecal P excretions, but increased urinary P excretions. They also observed that ileal digestibility of P was increased with increasing phytase supplementation Murry (1995) noticed a phytase interaction on P digestibility, whereas in the present study no interaction between P digestibility and phytase supplementation was evident from the statistical analysis.

5.1.7.3 Magnesium Availability

Per cent availability of Mg of the experimental rations T1 to T8 was 71.06, 66.90, 65.42, 68.27, 62.85, 68.05, 65.51 and 66.92 respectively. On statistical analysis no significant differences (P>0.05) were evident between the treatment groups. Also no interaction (P>0.05) between the levels of Ca, P or phytase was seen significant with regard to Mg availability. In contrast to the findings of the present study Veum *et al.* (2006) observed that pigs fed barley based diets containing low P, supplemented with *E. coli* phytase had greater per cent apparent absorption of Mg than pigs fed the positive control diets.

Viswanathan *et al.* (2007) also could not note any effect on digestibility of Mg as a result of citric acid and phytase supplementation which also agrees with the results of the present study.

5.1.7.4 Zinc Availability

In the present study the per cent availability of Zn recorded for the eight treatment groups T1, T2, T3, T4, T5, T6, T7 and T8 were 70.70, 66.94, 71.68, 66.08, 67.33, 71.46, 69.36 and 67.98 respectively. Statistical analysis revealed that dietary Ca, P and phytase had no effect on Zn availability. There was no interaction between the levels of Ca, P or phytase.

In contrast to these findings Adeola *et al.* (1995) indicated that daily Zn retention was increased when phytase was supplemented to diets regardless of dietary Zn level. Viswanathan *et al.* (2007) also observed a higher apparent digestibility of Zn for rations supplemented with citric acid and phytase. But Veum *et al.* (2006)

observed in pigs fed barley based diets containing low P supplemented with *E. coli* phytase that addition of *E.coli* phytase did not increase the apparent percentage absorption of Zn which is in concurrence with the present study.

5.1.7.5 Copper Availability

The per cent Cu availability of the eight experimental rations T1 to T8 of the present study were 64.18, 64.46, 67.97, 75.94, 68.46, 67.09, 60.41 and 69.03, respectively. On statistical analysis of the data, it was found that the dietary levels of Ca, P or phytase did not affect ($P>0.05$) the availability of Cu in the present study. However the interaction between the dietary Ca and P level was found significant ($P<0.05$) for Cu availability. Ration containing low level of Ca and high level of P (T4) had higher ($P<0.05$) Cu availability than the other rations. No other interactions were found significant.

Adeola *et al.* (1995) and Kies *et al.* (2006) found that apparent Cu balance was improved with phytase addition. Viswanathan *et al.* (2007) also observed a better apparent digestibility of Cu in citric acid supplemented groups of pigs.

Veum *et al.* (2006) observed that addition of *E.coli* phytase did not increase the apparent absorption of Cu which is in accordance with that of the present study.

5.1.7.6 Manganese Availability

In the present study the per cent availability of Mn recorded for the eight treatment groups was 73.47, 62.85, 75.61, 65.93, 71.11, 68.90, 63.62 and 70.75, respectively (Table 13). The statistical analysis of the data revealed no significant effect ($P > 0.05$) of dietary levels of Ca, P and phytase on the availability of Mn. It was also observed that there was a significant interaction ($P< 0.05$) between the dietary Ca and phytase level for Mn availability.

Viswanathan *et al.* (2007) observed a better apparent digestibility of Mn in citric acid plus phytase supplemented groups.

5.1.8 Plasma Biochemical Parameters

5.1.8.1 Calcium

The data on plasma biochemical parameters from experimental animals collected on the 85th day of the experiment are shown in Table 14. Plasma Ca concentration of the animals belonging to groups T1, T2, T3, T4, T5, T6, T7, T8 were 8.8, 9.2, 8.9, 8.9, 9.1, 9.2, 8.5 and 8.5 mg/dl initially and the values for plasma Ca was 11.0, 11.5, 10.02, 11.94, 10.43, 10.99, 11.80 and 11.92 mg/dl for blood collected on 85th day of the experiment. The statistical analysis of the data revealed no significant difference ($P > 0.05$) in blood Ca levels due to different dietary Ca levels (T1, T2, T3 and T4 vs T5, T6, T7 and T8) and P (T1, T2, T5, T6 vs T3, T4, T7 and T8) levels while the effect of phytase on blood Ca level (T1, T3, T5 and T7 vs T2, T4, T6 and T8) was significant ($P < 0.01$).

Two way analysis of the data on blood Ca level revealed significant interaction ($P < 0.01$) between dietary Ca and P levels. Pigs fed ration with 1 per cent Ca and 0.6 per cent P (T7) showed significantly higher blood Ca level than those fed T3 (0.6 per cent Ca and 0.6 per cent P) and T5 (1 per cent Ca and 0.3 per cent P).

Interaction between dietary Ca, P and phytase on blood Ca level was found nonsignificant ($P > 0.05$).

Reinhart and Mahan (1986) and Qian *et al.* (1996) found that serum Ca concentrations were higher and serum P concentrations were lower with increasing dietary Ca:P ratio, with the response was more pronounced at low dietary P levels in grower and finisher swine. In the present study also statistical analysis was suggestive of interaction between Ca and P levels in the diet on plasma Ca concentration.

Adeola *et al.* (1995) and Moreira *et al.* (2003) observed that plasma Ca concentration was not significantly affected by dietary phytase in pigs in contrast to the observations of the present study in which significant effect of phytase was seen on plasma Ca concentration.

5.1.8.2 Phosphorus

Plasma P concentrations of the animals belonging to groups T1 to T8 were 6.0, 5.13, 5.37, 5.1, 6.1, 4.83, 4.5 and 6.2 mg/dl respectively initially and were 5.65, 5.11, 5.17, 5.03, 5.68, 5.31, 5.06 and 6.00 mg/dl, respectively on 85th day of the experiment. On statistical analysis of the data, it could be noticed that there was significant difference (P< 0.01) in blood P levels due to different dietary Ca (T1, T2, T3 and T4 vs T5, T6, T7 and T8) and P (T1, T2, T5, T6 vs T3, T4, T7 and T8) levels. Pigs fed rations T1 and T8 (0.6 per cent Ca and 1 per cent Ca) and T1 and T5 (0.3 per cent P each) and T8 (0.6 per cent P) registered significantly higher (P< 0.01) plasma P concentration compared to that of the other groups.

Two way analysis of the data on blood P level revealed significant interaction (P< 0.01) between dietary Ca and P levels. Pigs fed rations with 1 per cent Ca and 0.3 per cent P (T5) and 1 per cent Ca and 0.6 per cent P (T7) showed significantly higher (P< 0.01) blood P levels than those fed rations with 0.6 per cent Ca and 0.3 per cent P (T1) and 0.6 percent Ca and 0.6 per cent P (T3). Two way significant (P<0.01) interaction was also noticed between dietary Ca and phytase levels on plasma P levels. Pigs fed rations with 0.6 per cent Ca supplemented with phytase (T2 and T4) showed significantly (P< 0.01) lower blood P level than T1 (0.6 per cent Ca without phytase) whereas those fed T8 (1 per cent Ca with supplemented phytase) showed significantly (P<0.01) higher blood P level than T7 (1 per cent Ca without phytase supplementation).

Cromwell *et al.* (1970) reported that by increasing the dietary P from 0.34 to 0.56 per cent to grower and from 0.29 to 0.44 per cent to finisher resulted in a linear increase in serum P where as in the present study there was a decrease in plasma P with increase in dietary P without phytase supplementation. Kornegay *et al.* (1981b) observed that serum P of barrows, boars and gilts were reduced when less than NRC suggested levels of P were fed in contrast to the present study.

Kornegay *et al.* (1981a) observed that serum P was decreased as dietary Ca and P increased and this finding is in concordance with the present results obtained for plasma P levels in pigs. Qian *et al.* (1996) also observed decreased plasma P concentration when the Ca:tP ratio was increased in pigs.

The effect of phytase on plasma P level (T1, T3, T5 and T7 vs T2, T4, T6 and T8) was not significant (P >0.05) in the present study. However the two way analysis revealed significant interaction between dietary P and phytase levels on plasma P levels. Pigs fed rations with low P without supplemented phytase (T1 and T5) and with high P with supplemented phytase (T8) showed significantly higher (P<0.01) plasma P levels than those fed rations with low P with phytase (T1 and T2) and high P without .phytase (T7 and T3).

Interaction between dietary Ca, P and phytase also was found significant (P< 0.01) in the present study.

Lei *et al.* (1993), Han *et al.* (1997), Liu *et al.* (1997), Martinez *et al.* (2004) and Jendza *et al.* (2005) observed that supplemental phytase significantly enhanced plasma concentrations of iP in pigs which is in agreement with that of the present study where supplemental phytase increased plasma P concentration in rations containing high Ca and P .

However Moreira *et al.* (2003) observed that phytase levels did not affect plasma Ca and P or Ca:P ratio.

5.1.8.3 Magnesium

The initial plasma Mg concentrations of the animals belonging to the eight dietary treatments were 2.1, 2.2, 1.9, 1.9, 2.1, 2.2, 1.9 and 2.1 mg/dl and the values corresponding to the final blood collection (85th day) were 1.91, 1.95, 1.93, 1.92, 1.84, 1.83, 1.95, 1.86 mg/dl. No significant effects or interactions (P>0.05) were seen for plasma Mg concentration with dietary Ca, P or phytase levels.

Kornegay *et al.* (1981a) and Qian *et al.* (1996) in concurance with the present result observed no difference in serum Mg, when dietary Ca and P were increased whereas Adeola *et al.* (1995) found that plasma Mg concentration was increased in response to the addition of phytase to the diet.

5.1.8.4 Zinc

Plasma Zn values corresponding to the initial blood collected from the animals belonging to the eight dietary treatment groups were 0.95, 0.85, 0.85, 0.96, 1.0, 1.25, 1.35 and 0.99 ppm while that of the final collection were 1.3, 0.95, 1.17, 1.18, 0.98, 1.12, 0.95 and 0.99 ppm respectively. Statistical analysis of the data indicated that different dietary Ca levels (T1, T2, T3 and T4 vs T5, T6, T7 and T8) had a significant (P<0.01) effect on plasma Zn concentration. Rations with high Ca levels (T5, T6, T7 and T8) registered significantly low plasma Zn concentrations. The data revealed no significant effect of dietary P level on plasma Zn concentration. (T1, T2, T5, T6 vs T3, T4, T7 and T8).

Two way interaction was seen significant (P<0.01) in the present study between dietary Ca and P on plasma Zn concentration. Pigs fed ration T3 with low Ca and high P (0.6 per cent Ca and 0.6 per cent P) showed significantly (P<0.01) high plasma Zn concentration compared to rations T1 (0.6 per cent Ca and 0.3 per cent P), T5 (1 per cent Ca and 0.3 per cent P) and T7 (1 per cent Ca and 0.6 per cent P).

There was also significant two way interaction seen between dietary levels of both Ca and phytase (P<0.05) and P and phytase (P<0.01) respectively. Pigs fed ration with high Ca without phytase (T5 and T7), showed significantly (P<0.05) low plasma Zn concentration when compared to those fed rations containing low level of Ca without phytase supplementation (T1 and T3) and also rations containing low level of Ca with phytase (T2 and T4). Two way interaction between dietary P levels and phytase addition was also seen significant (P<0.01) for plasma Zn. Feeding pigs with low P plus phytase rations (T2 and T6) showed significantly (P<0.01) low plasma Zn concentration compared to those fed rations with low P without phytase supplementation (0.3 per cent P), (T1 and T5) and that containing high dietary P, (T3 and T7) without phytase supplementation and ration containing high dietary P with phytase supplementation.

A three way interaction ($P<0.01$) was also noticed between dietary Ca, P and phytase levels on plasma Zn concentration in the present study.

Lei *et al.* (1993) observed that either supplemental phytase or supplemental Zn increased plasma Zn concentrations in weanling pigs. In the present study supplemental phytase increased the plasma Zn in diet containing low Ca than in diet containing high Ca. Adeola *et al.* (1995) noticed that plasma Zn concentration increased when phytase was added to the diet containing no supplemental Zn, but plasma Zn concentration was not affected by phytase, when the .diet was supplemented with Zn.

Qian *et al.* (1996) observed that plasma Zn was not influenced by dietary Ca:tP ratio. But in the present study there was a two way interaction noticed between Ca and P levels of feed, both dietary Ca and P levels and level of phytase supplementation affecting plasma Zn concentration.

5.1.8.5 Copper

The plasma concentrations of Cu recorded for the animals belonging to the eight dietary groups were 1.15, 1.30, 0.99, 1.15, 1.2, 1.2, 1.0 and 1.1 ppm and the final values were 1.12, 1.28, 1.47, 1.57, 1.33, 1.50, 1.53 and 1.72 ppm. On statistical analysis, significant ($P<0.01$) effect of dietary levels of Ca, P and phytase was observed for plasma Cu concentration. Pigs fed rations (T5, T6, T7 and T8) containing high Ca levels showed significantly ($P<0.01$) higher plasma Cu concentrations than those fed rations containing low Ca (T1, T2, T3 and T4). Similarly feeding of rations containing low P (T1, T2, T5 and T6) showed significantly ($P<0.01$) lower plasma Cu concentration compared to those fed rations containing high dietary P (T3, T4, T7 and T8). Pigs fed Phytase supplemented rations had significantly higher plasma Cu concentrations (T2, T4, T6 and T8) compared to the unsupplemented rations (T1,T3, T5 and T7).

Pond *et al.* (1978) reported that diets severely deficient in Ca can be tolerated by the finishing pig and growing rat without drastic effect on net uptake of tissue Ca, Cu, Fe, Mg, Mn and Zn even in association with wide variations in diet Cu and Zn levels. Murry *et al.* (1997) observed no consistent effect of dietary microbial phytase for serum Cu concentrations in pigs fed supplemental phytase while in the present study it was observed that phytase supplementation significantly increased the plasma Cu concentration of the pigs.

5.1.8.6 Manganese

Initial plasma concentration of Mn of animals belonging to the eight dietary groups was 0.01, 0.01, 0.01, 0.01, 0.02, 0.01, 0.01 and 0.02 ppm and the values corresponding to the final collection was 0.01 ppm in all the eight dietary groups. On statistical analysis of the data there was no effect of dietary Ca, P levels and phytase supplementation on plasma Mn concentration.

Pond *et al.* (1978) reported that diets severely deficient in Ca can be tolerated by the finishing pig and growing rat without affecting net uptake of tissue Ca, Cu, Fe, Mg, Mn and Zn even in association with wide variations in diet Cu and Zn levels.

5.1.8.7 Alkaline Phosphatase (ALP)

In the present study initial plasma alkaline phosphatase enzyme activity varied between 225 to 280 U/L and corresponding values obtained during the final collection of blood (85^{th} day) were 334, 355, 298, 337, 317, 364, 325 and 357 respectively U/L respectively.

Statistical analysis of the data revealed that the levels of Ca, P and phytase in the diets significantly ($P<0.05$) affected the plasma ALP activity. Pigs fed rations containing high Ca (T5, T6, T7 and T8) registered significantly ($P<0.05$) higher plasma ALP activity than those containing low dietary Ca level. Feeding of ration containing low Ca and high P (0.6 per cent Ca and P) registered lower plasma ALP activity than those fed rations containing low level of Ca and low level of P (T1 and

T2). All the phytase supplemented rations showed significantly (P<0.05) higher ALP activity in pigs.

Two way interaction between the dietary Ca and P levels were found significant (P<0.05) for the plasma ALP activity. Pigs fed rations containing high dietary P levels with high Ca levels (T7 and T8) showed better plasma ALP activity than those with low Ca rations (T3 and T4). Two way interaction between Ca and phytase levels were found significant (P<0.05) for the plasma ALP activity. Pigs fed ration containing low Ca without phytase (T1 and T2) showed significantly (P<0.05) low plasma ALP activity than rations with low Ca and with phytase (T2 and T4). Similarly rations with high Ca without phytase supplementation showed significantly (P<0.05) low plasma ALP activity.

A significant three way interaction (P<0.05) between dietary Ca, P and phytase levels was noted for the plasma ALP activity in the present study.

Hoefer *et al.* (1960) observed increased serum ALP activity in pigs due to dietary supplementation of Zn to control rations varying in Ca levels in pigs. Hoekstra *et al.* (1967) and Lei *et al.* (1993) observed significantly higher activities of serum ALP than that of unsupplemented group similar to the results of the present study. Boyd *et al.* (1983) found that the plasma ALP increased with time and with the concentration of tP (0.26 to 0.50 per cent) indicating ALP as a sensitive index in P availability studies. Qian *et al.* (1996) on the contrary observed that ALP activity was not influenced by Ca:tP ratio.

Han *et al.* (1997) noted a lower serum ALP activity in pigs fed diets supplemented with cereal or microbial phytase compared to those fed control diets whereas phytase supplemented groups had higher plasma ALP activity in the present experiment.

5.1.9 Economics

Data on total body weight gain, total feed intake, total feed cost and cost of feed/kg gain of pigs maintained on the eight dietary treatments T1, T2, T3, T4, T5, T6, T7 and T8 are presented in Table 15. The cost of ingredients used for the study was as per the rate contract fixed by the College of Veterinary and Animal Sciences Mannuthy, for the year 2006-07. Phytase was supplied by Polchem Hygiene Laboratories PVT. Ltd, Pune at the rate of Rs.265 /kg. Cost of feed per kg body weight gain of pigs maintained on the eight dietary treatments were Rs.59.15, 60.19, 60.58, 62.77, 62.12, 60.71, 62.69 and 63.53 respectively. It was observed that cost of feed/kg gain was lowest for the ration T1 among the eight rations.

The overall results obtained in the first experiment can be summarized as follows. Feeding piglets with rations containing different levels of Ca (0.6 and 1 per cent), P (0.3 and 0.6 per cent) and phytase (0 and 750 U/kg) did not affect growth rate, feed efficiency, DM intake, CP and NFE digestibility and P, Mg, Zn, Cu and Mn availability. Rations T3 (0.6 per cent Ca and 0.6 per cent P), T5 (1 per cent Ca and 0.3 per cent P) and T7 (1 per cent Ca 0.6 per cent P) had lower DM digestibility than rations T1, T2, T4, T6 and T8. Rations T4 and T8 had higher CF digestibility while rations T7 and T8 had lower EE digestibility than the rest of the rations. Feeding of phytase supplemented rations (T2, T4, T6 and T8) increased Ca availability and plasma Ca levels than those fed the rest of the rations. Pigs fed rations T1, T5 and T8 had higher plasma P than those of the other treatment groups. The cost of feed / kg gain was lowest for T1.

Since the ration T1 with low Ca, low P and without phytase gave similar growth and feed efficiency with lowest cost of production, this ration was taken as the control ration for the second experiment.

5.2 EXPERIMENT 2

5.2.1 Chemical Composition of Experimental Ration

The percentage chemical composition of the grower rations are presented in Table 16 and that of finisher rations in Table 17.

The CP of the three grower rations was 18.43, 18.48 and 18.37 per cent and that of finisher ration was 16.15, 16.32 and 16.63 per cent, respectively. As per ICAR feeding standards ration for pigs weighing from 10 to 40 kg should contain 16 per cent CP and that of pigs weighing above 40 kg should contain 14 per cent CP. As per NRC (1998) the grower and finisher ration should contain 18 and 16 per cent CP respectively. The calculated ME value of the grower and finisher feeds were 3329 and 3349 kcals /kg, respectively which is in accordance with NRC (1998) recommendations of 3265 kcals ME/ kg feed. According to ICAR (1985), the feed for growing and finishing swine should contain 68 per cent TDN and 3000 kcals of digestible energy /kg feed.

5.2.2 Mineral Composition

The mineral composition of both grower and finisher rations is given in Table 16 and 17, respectively. The Ca content of the three grower rations was 0.75, 0.2 and 0.19 per cent respectively. Similarly the P content of the feeds was 0.57, 0.25 and 0.24 per cent respectively. The Ca content of finisher ration was 0.75, 0.20 and 0.20 per cent respectively and P content was 0.56, 0.26 and 0.25 per cent respectively.

As per ICAR (1985), the Ca and P requirement for pigs weighing from 10 to 40 kg are 0.6 and 0.5 per cent respectively and from 40 kg onwards, 0.5 and 0.4 per cent respectively. The Ca and P requirements are also 0.6 and 0.5 per cent for growers and 0.5 and 0.45 per cent for finishers respectively, as per NRC (1998).

Out of the three rations of the present study only the control ration T1 met the Ca and P requirements as per ICAR (1985) and NRC (1998). In rations T2 and T3 the Ca and P contents were lower than the requirement values since no mineral supplements were added in these rations. This was done to similate the condition in the field where the pigs are reared exclusively on kitchen / hotel waste and to study the effect of phytase supplementation on the utilization of minerals that are present in the feed ingredients used in the ration.

Magnesium requirement of grower and finisher rations is 0.04 per cent (NRC 1998), but the Mg content of the three dietary treatments was higher both for grower (0.33, 0.24 and 0.24 per cent) and finisher (0.34, 0.24 and 0.25 per cent) rations. Zinc, Cu and Mn requirements are 80, 5 and 3 ppm for growers and 50, 4 and 2 ppm, respectively for finishers (NRC 1998). The concentrations of Zn in the three grower rations T1, T2 and T3 were 260.03, 36.39 and 37.81 ppm and in the finisher rations 336.47, 44.18 and 42.9 ppm respectively. The Cu content was 9.59, 9.52 and 9.03 ppm and 9.40, 9.05 and 9.8 ppm, respectively in the grower and finisher rations. The Mn contents were 13.79, 13.44 and 12.98 ppm in grower rations and 13.16, 13.58 and 12.44 ppm respectively in the finisher rations.

5.2.3 Body Weight

The body weight of pigs under the three dietary treatments T1, T2 and T3 recorded at fortnightly intervals are presented in Table 18 and graphically represented in Fig.7. Average final body weight of pigs of the three dietary treatments were 67.79, 69.54, 64.75 kg, respectively. Average total body weight gain of animals belonging to groups T1, T2 and T3 were 59.12, 60.58, 55.66 kg respectively. The statistical analysis of the data revealed significant difference ($P < 0.05$) between the treatment groups. There was no significant difference ($P > 0.05$) with regard to body weight of pigs of treatments T1, T2 and T3 in 1st , 2nd, 3rd and 5th fortnight. Further, body weight of pigs belonging to T2 (phytase supplemented ration) was significantly higher ($P < 0.05$) than that of T3 in fortnights 4, 6, 7, 8 and 9. There was no significant difference between body weights of T1 and T2 (control ration and ration supplemented with phytase alone) in fortnights 4, 6, 7, 8 and 9. The results of the present study revealed that phytase supplementation significantly increased the total weight gain of the animals fed with a ration without any mineral supplementation and the weight gain of these animals reached up to the level of those fed the control ration supplemented with adequate levels of Ca and P.

The results of the present study are in agreement with Cromwell *et al.* (1993), Lei *et al.* (1993), Adeola *et al.* (1995) and Biehl *et al.* (1996) who obtained increased body weight as a result of phytase supplementation. Similarly Harper and Kornegay (1996), Harper *et al.* (1997), Liu *et al.* (1997), Murry *et al.* (1997) and O'Quinn *et al.* (1997) observed linear increase in growth performance in pigs when graded levels of phytase was supplemented to low P diets.

Hariharan (2003) observed increased body weight in broiler chicks with addition of citric acid or phytase or their combination to low aP diets. Sakthivel (2003) reported total weight gain of 55.93, 53.33 and 55.10 kg in pigs fed rations containing dried cuttle fish waste silage replacing dried fish at 0, 50 and 100 per cent, respectively. In the present study the total weight gain obtained were similar or even better. Sekhar (2003) reported body weight gain of 52.8, 57.4, 60.8 kg in swine in concurrence with the values obtained during the present study.

Dilger *et al.* (2004) and Onyango *et al.* (2004) observed similar weight gain in broiler chicks on phytase supplementation. Pillai *et al.* (2006) observed that increasing iP resulted in a linear increase in weight gain and adding *E. coli* phytase resulted in a linear and quadratic increase in weight gain.

5.2.4 Average Daily Gain

Data on ADG of pigs under the three dietary treatments T1, T2 and T2 are presented in Table 19 and are graphically represented in Fig. 8. For the three dietary treatments T1, T2 and T3, the values for cumulative ADG were 0.51, 0.52 and 0.48 kg respectively. The statistical analysis revealed that there was significant difference ($P<0.05$) between the treatment groups with regard to ADG. There was no difference ($P>0.05$) with regard to ADG between those of treatments T1 and T2 and T1 and T3, but the ADG was higher ($P< 0.05$) for T2 than that of T3. The result of the present study revealed that phytase supplementation increased ADG of animals fed with a ration without any mineral supplementation.

Increased ADG as a result of phytase supplementation was reported by Han *et al.* (1997), Matsui *et al.* (2000), Landblom *et al.* (2002), Gentile *et al.* (2003), Augspurger *et al.* (2004), Omogbenigun *et al.* (2004), Shelton *et al.* (2004), Jendza *et al.* (2005), Kies *et al.* (2005) and Brana *et al.* (2006) which agrees well with the results in the present study. Veum *et al.* (2006) and Guy *et al.* (2008) also reported similar results with phytase supplementation.

Kornegay *et al.* (1981), Nimmo *et al.* (1981) and Crenshaw (1986) in their experiments with different levels of Ca and P opined that ADG in pigs was not affected by higher levels of Ca and P. No significant difference in ADG as a result of phytase supplementation was reported in pigs by Kemme *et al.* (1997a), Radcliffe *et al.* (1998), Moreira *et al.* (2003), Park *et al.* (2003) and Cervantes *et al.* (2004) and in broilers by Akyurek *et al.* (2005).

Williams *et al.* (2005) on the contrary observed that dietary phytase decreased ADG in pigs fed diets containing1000 or 2000 ppm of Zn in pigs.

The ADG values obtained in the present experiment are higher than that reported by Sekhar (2003) who reported 352, 383 and 405 g in pigs supplemented with bakers yeast.

5.2.5 Dry Matter Intake

Data on total DMI of animals belonging to the three dietary groups are given in Table 19 and Fig.9, the values being 334.04, 334.09 and 322.29 kg, respectively. On statistical analysis there was no significant difference (P>0.05) between the treatment groups with regard to DMI indicating that phytase supplementation and Ca and P level in the ration had no effect on DMI. This is in agreement with Kornegay *et al.* (1981a) who found no overall difference in daily feed intake between boars fed diets containing Ca and P at 100, 125 and 150 per cent of NRC recommendations. O'Quinn *et al.* (1997) also observed that decreasing dietary phosphorus content by 25 per cent of NRC recommendations did not affect average daily gain (ADG) and feed intake during growth period.

On the other hand Pond *et al.* (1978) observed higher feed intake with high Ca diets. Lei *et al.* (1993), Liu *et al.* (1997,) Dilger *et al.* (2004) and Kies *et al.* (2006) also indicated that supplemental phytase significantly enhanced feed intake in pigs. Similarly Guy *et al.* (2008) observed that feed intake in weaner pigs was increased by phytase supplementation, either alone or in combination with organic acids.

Lower daily feed intake was reported by Nimmo *et al.* (1981) and Qian *et al.* (1996) in gilts fed low Ca:P diet or diet with wider Ca:P ratio.

No effect on average feed intake as a result of phytase supplementation was reported by Han *et al.* (1997) when they compared the effect of microbial phytase, cereal phytase or iP supplementation in pigs which agrees well with the results obtained in the present study. Moreira *et al.* (2003), Park *et al.* (2003), Shelton *et al.* (2004), Jendza *et al.* (2005), Kies *et al.* (2005) and Williams *et al.* (2005) also did not observe any effect on feed intake of pigs as a result of phytase supplementation. Onyango *et al.* (2004) and Akyurek *et al.* (2005) also observed that phytase supplementation did not affect feed intake in broilers while Xian *et al.* (2004) in rabbits.

5.2.6 Feed Conversion Efficiency

The feed conversion efficiency of pigs maintained on dietary treatments T1,T2 and T3 are presented in Table 19 and the data is presented in Fig. 10. Animals belonging to treatments T1, T2 and T3 registered a feed conversion efficiency of 5.66, 5.52 and 5.79, respectively. There was no significant difference ($P>0.05$) among the treatment groups.

Kornegay *et al.* (1981) Nimmo *et al.* (1981) and Crenshaw (1986) obtained similar results as in the present study, and observed no effect on feed : gain ratio in diets containing different Ca:P ratios. Reinhert and Mahan (1986) evaluated the effects of various Ca:P ratios at two dietary P levels for starter, grower and finisher diets and found that pigs fed low P diets displayed lower feed efficiency after the

Ca:P ratio exceeded 1.3:1, whereas negative effects of feeding high dietary P levels were exhibited only when the Ca:P ratio exceeded 2.0:1. Cera and Mahan (1988) also observed no difference in feed:gain ratios between the rations containing different levels of Ca and P as observed in the present study.

Han *et al.* (1997) obtained similar feed efficiency in pigs when supplemented with microbial phytase, cereal phytase or iP indicating that supplementation of phytase can completely replace iP in the diet of pigs. No effect of phytase supplementation on feed conversion was reported in growing pigs by Kemme *et al.* (1997), O'Quinn *et al.* (1997), Moreira *et al.* (2003), Cervantes *et al.* (2004) and Martinez *et al.* (2004) in concurrence with the present study.

Boling *et al.* (2001) observed that phytase addition had no significant effect on PER values for any of the ingredients evaluated, except for casein in chicks, Hariharan (2003) also observed no significant increase in feed efficiency and protein efficiency in broiler chicks fed diets with citric acid or phytase. These results are in agreement with the results of the present study

Mavromichalis *et al.* (1999) found that removing vitamin and trace mineral premixes from diets during late finishing had no effect on gain:feed ratio and this is in concurrence with the present finding. Hastad *et al.* (2004), on the other hand suggested that complete removal of supplemental P in diets for finishing pigs decreased gain:feed ratio.

Lei *et al.* (1993), Cromwell *et al.* (1993), Adeola *et al.* (1995), Biehl *et al.* (1996), Qian *et al.* (1996), Liu *et al.* (1997), Murry *et al.* (1997), Liu *et al.* (1998), Radcliffe *et al.* (1998), Sands *et al.* (2001), Park *et al.* (2003), Williams *et al.* (2005) and Brana *et al.* (2006) also observed improved gain:feed ratio with phytase supplementation in pigs.

5.2.7 Digestibility of Nutrients

5.2.7.1 Dry Matter

Chemical composition of faeces of pigs fed with different experimental diets is shown in Table 20. Data on per cent digestibility of nutrients are presented in Table 21 and is graphically represented in Fig. 11. The percentage DM digestibility of the three rations T1, T2 and T3 were 86.11, 87.92 and 87.95. On statistical analysis it was seen that the DM digestibility of rations T2 and T3 was higher ($P<0.05$) than that of T1.

Harper *et al.* (1997), O'Quinn *et al.* (1997), Park *et al.* (2003), Cowieson and Adeola (2005) and Jendza *et al.* (2005) noticed that apparent digestibility of DM was not affected by phytase supplementation in swine whereas similar results were reported by Dilger *et al.* (2004), Akyurek *et al.* (2005) and Onyango *et al.* (2005) in broilers. Anaya *et al.* (2008) also observed that the DM digestibility was not affected when phytase was added to soyabean meal based commercial dog feed.

On contrary, Mroz *et al.* (1994) observed significant enhancement of apparent digestibility of DM in pigs fed corn-tapioca-soyabean meal diet supplemented with microbial phytase. Murry *et al.* (1997) noted that adding phytase increased apparent digestibility of DM quadratically in the adequate P diets. Sands *et al.* (2001) also observed that phytase addition resulted in higher DM digestibility in pigs fed the normal corn diets.

Johnston *et al.* (2004), Omogbenigun *et al.* (2004), Juanpere *et al.* (2005), Kies *et al.* (2005), Kies *et al.* (2006) and Viswanathan *et al.* (2007) also observed higher digestibility of DM when supplemented with phytase in pigs.

But Kemme *et al.* (1997) observed that addition of phytase to the diet significantly reduced the digestibility of DM in piglets but not in growing-finishing pigs and sows fed corn-soyabean meal based diets.

5.2.7.2 Crude Protein

The results of the present digestibility study revealed per cent digestibilities of 83.63, 85.31 and 84.97 for crude protein for the three rations T1, T2 and T3 respectively. The data are shown in Table 21 and are graphically represented in Fig. 11. There was no significant difference (P>0.05) among the treatment groups on statistical analysis. It could be concluded that phytase supplementation to rations with or without added minerals had no effect on CP digestibility.

O'Quinn *et al.* (1997) observed in growing-finishing pigs fed sorghum-soyabean meal based diets that decreasing dietary P did not affect apparent digestibility of N. O'Quinn *et al.* (1997), Traylor *et al.* (2001) and Cervantes *et al.* (2004) also did not observe any effect of phytase supplementation on the apparent ileal digestibility of CP and amino acids in pigs fed sorghum-soyabean meal diet. No effect of phytase on the apparent total tract digestibility of CP was reported by Liao *et al.* (2005) and Jendza *et al.* (2005) and these findings agree well with the finding in the present study. Akyurek *et al.* (2005) in a study noted that phytase supplementation to corn based broiler diets containing low aP registered similar CP digestibility when compared with diets containing adequate levels of aP.

On the other hand, Mroz *et al.* (1994), Murry *et al.* (1997), Johnston *et al.* (2004), Omogbenigun *et al.* (2004) and Kies *et al.* (2005) noted that adding phytase increased CP digestibility in weaned pigs. Onyango *et al.* (2005) observed that an evolved *E. coli* phytase improved retention of N and amino acids such as arginine, histidine, threonine, tryptophan, valine, aspartate and proline in adequate P fed chicks whereas apparent ileal digestibility of N was not affected by phytase supplementation to low P diet.

Ravindran *et al.* (1999) observed that addition of microbial phytase to broiler diets improved the digestibility of protein and amino acids in all feed stuffs tested.

Thomas (2005) reported CP digestibility values of 69 and 65 per cent respectively for chromium supplemented and control ration fed pigs which are lower than the values obtained in the present study.

5.2.7.3 Ether Extract

The digestibility percentages of EE in animals fed the three treatment rations were 52.81, 53.89 and 53.89 respectively. The data are shown in Table 21 and are graphically represented in Fig. 11. There was no significant difference (P>0.05) evident between the three treatment groups on statistical analysis. It could be concluded that phytase supplementation to rations without any mineral supplementation had no effect on EE digestibility.

In contrast with the present observation Kies *et al.* (2005) observed that 1500 PU of phytase/kg feed increased apparent digestibility of fat by 1.2 per cent. Viswanathan *et al.* (2007) also observed that the digestibility coefficient of EE was significantly higher for citric acid and citric acid plus phytase supplemented groups than that of the control, in Large White Yorkshire pigs. Akyurek *et al.* (2005) also reported that phytase supplementation to corn based broiler diets containing low aP registered improved ileal digestibility of EE.

Thomas (2005) reported lower digestibility values of 35 and 28 per cent for chromium incorporated and control ration respectively and these values are lower than that obtained in the present study.

5.2.7.4 Crude Fibre

The per cent digestibility of CF of the three treatments T1, T2 and T3 were 55.49, 57.41 and 55.94, respectively. The data are shown in Table 21 and graphically represented in Fig. 11. On statistical analysis there was no significant difference (P>0.05) between the treatment groups indicating that the supplementation of phytase to rations without added minerals had no effect on CF digestibility.

Sakthivel (2003) reported per cent digestibility values of 18.63, 12.39 and 24.60 for crude fibre in pigs fed control diet and cuttle fish waste silage at two different levels. Sekhar (2003) reported crude fibre digestibility value of 25.52 and 32.82 for the 0.25 and 0.5 per cent bakers yeast supplemented diets respectively and 22.26 per cent for the control diet. Similar crude fibre digestibility value of 52 per cent as that of the present study was reported by Thomas (2007) of chromium incorporated diets fed to pigs.

Viswanathan *et al.* (2007) observed that the digestibility coefficient of crude fibre was significantly higher for citric acid and citric acid plus phytase supplemented groups than that of control. In the present trial also phytase supplemented animals registered higher crude fibre digestibility though was not statistically significant.

5.2.7.5 Nitrogen Free Extract

The results of the present study registered per cent digestibility of 92.22, 93.55 and 93.56 respectively for NFE and it was found that significant difference (P< 0.05) existed between the treatment groups with regard to NFE digestibility. The data are shown in Table 21 and graphically represented in Fig.11. It was noted on statistical analysis that there was no significant difference (P>0.05) between the treatments and it could be concluded that phytase supplementation to rations without any added minerals did not have any effect on digestibility of NFE. Sakthivel (2003) reported NFE digestibility values of 74.17, 73.55 and 75.89 per cent respectively in pigs fed control diet and cuttle fish waste silge at two different levels. Sekhar (2003) also reported similar NFE digestibility values of 75.03 and 76.27 per cent for 0.25 and 0.5 per cent bakers yeast supplemented diets respectively while Thomas (2007) observed 71 and 70 per cent digestibilities for NFE in pigs fed control and chromium supplemented rations.

5.2.8 Availability of Minerals

The data on the availability of different minerals are shown in Table 22 and depicted in Fig.12.

5.2.8.1 Calcium Availability

In the present study the percentage availability of Ca for the three rations T1, T2 and T3 were 52.03, 64.68 and 59.88 respectively. The data on statistical analysis showed that there was significant difference ($P<0.01$) between the dietary treatment groups with regard to Ca availability. Calcium availability of the rations T2 and T3 was higher ($P<0.01$) than ration T1 while there was no difference between rations T2 and T3. Similar results were noted by Lei *et al.* (1993), Mroz *et al.* (1994), Adeola *et al.* (1995), Murry (1995) and Kemme *et al.* 1997) and they observed increased Ca digestibility for rations with dietary microbial phytase supplementation. Liu *et al.* (1997), Murry *et al.* (1997) and O'Quinn *et al.* (1997) also noted that supplemental phytase increased Ca absorption in pigs.

Sauer *et al.* (2003), Johnston *et al.* (2004) and Veum *et al.* (2006) also observed improved Ca digestibility and absorption along with phytase supplementation in pigs whereas Akyurek *et al* (2005) could observe that phytase supplementation at 0.5 g/kg to broiler diets increased Ca and P retention. Xian *et al.* (2004) also observed the dietary phytase remarkably increased Ca retention in rabbits.

In contrast to the findings of the present study Liu *et al.* (2000) found that lowering the dietary Ca : tP ratio in the diets containing phytase did not affect Ca absorption.

5.2.8.2 Phosphorus Availability

Data on percentage availability of P of the three experimental rations T1, T2, T3 were 50.07, 58.05 and 52.45 respectively (Table 22 and Fig.12). On statistical analysis it was observed that there was significant difference ($P<0.05$) between the treatment groups with regard to P availability. It was seen that ration T2 showed significant increase ($P<0.05$) in P availability than that of T1 and there was no significant difference ($P>0.05$) between T1 and T3 and also between T2 and T3. It could be concluded from the present study that phytase supplementation of rations with unsupplemented minerals resulted in significant (P<0.05) increase in P availability.

In concurrence with the present study Lei *et al.* (1993), Mroz *et al.* (1994), Adeola *et al.* (1995), Murry (1995), Harper and Kornegay (1996), Harper *et al.* (1997) and O'Quinn *et al.* (1997) reported increased digestibility of P with phytase addition to low P diet in swine. Han *et al.* (1997) observed that the retention of total ingested P increased in pigs fed rations supplemented with microbial phytase and iP. Kemme *et al.* (1997) also observed that, irrespective of piglet weight, phytase supplementation enhanced the digestibility of P. Murry *et al.* (1997) and Liu *et al.* (1997) noted in nursery pigs that increasing levels of microbial phytase to low P and adequate P diets increased P absorption and retention.

O'Quinn *et al.* (1997) could notice that supplementing phytase at increasing levels reduced fecal P excretions, but increased urinary P excretions. They also observed that ileal digestibility of P was increased with increasing phytase supplementation. Similarly Liu *et al.* (1998), Liu *et al.* (2000) and Matsui *et al.* (2000) found that lowering the dietary Ca: tP ratio in the diets containing phytase linearly increased the apparent absorption of P.

Increased P digestibility and retention as a result of phytase supplementation was reported by Gentile *et al.* (2003), Park *et al.* (2003), Sauer *et al.* (2003), Johnston *et al.* (2004), Omogbenigun *et al.* (2004), Jendza *et al.* (2005), Kies *et al.* (2005), Brana *et al.* (2006), Nyachoti *et al.* (2006) and Veum *et al.* (2006) in pigs and Akyurek *et al.* (2005) in broilers and Xian *et al.* (2004) in rabbits.

5.2.8.3 Magnesium Availability

Availability of Mg from experimental rations T1, T2 and T3 were 68.15, 64.20 and 62.81 per cent respectively. On statistical analysis there was significant difference (P=0.01) between the three rations. It could be noted that rations T2 and T3 showed lower (P < 0.05) Mg availability than that of T1. There was no difference (P>0.05) between rations T2 and T3.

In contrast with the present study Veum *et al.* (2006) observed that pigs fed barley based diets containing low P supplemented with *E. coli* phytase had greater per cent apparent absorption of Mg than those fed the positive control diets while Viswanathan *et al.* (2007) could not note a better apparent digestibility of Mg in citric acid and phytase supplemented groups of Large White Yorkshire pigs, in accordance with the result of the present study.

5.2.8.4 Zinc Availability

From the data presented in Table 22 and Fig.12, it could be seen that the per cent availability of Zn for the experimental rations T1, T2, and T3 were 65.77, 64.93 and 61.78 respectively. On analysis of the data statistically there was significant difference with $P=0.08$ between the treatment groups with regard to Zn availability. On further analysis of the data it was evident that T3 ration was showing significantly lower ($P<0.05$) Zn availability than that of T1. Ration T2 had similar Zn availability ($P>0.05$) to that of T1 indicating that phytase supplementation improved availability of Zn that was present in the ingredients used in the ration.

In agreement to the results of the present study, Adeola *et al.* (1995) indicated that daily Zn retention was increased when phytase was added to diet regardless of dietary Zn level. Viswanathan *et al.* (2007) observed a higher apparent digestibility of Zn citric acid plus phytase supplemented groups.

Veum *et al.* (2006), on the contrary observed that addition of *E.coli* phytase did not increase the apparent percentage absorption of Zn in pigs.

5.2.8.5 Copper Availability

The per cent Cu availability of the three experimental rations of the present study were 67.60, 70.68 and 71.09 respectively. Statistical analysis of the data showed no significant difference (P>0.05) between the treatment groups with regard to Cu availability and that phytase supplementation had no effect on the Cu availability of the ration.

Veum *et al.* (2006) observed that addition of *E.coli* phytase did not increase the apparent percentage absorption of Cu which is in concurrence with the present study.

In contrast to the present study Adeola *et al.* (1995) found that apparent Cu balance was improved with phytase addition. Subsequently Kies *et al.* (2006) observed that digestibility of Cu was increased in weaner pigs with increasing phytase levels in digestible P deficient corn-barley soyabean meal diets. Viswanathan *et al.* (2007) observed a better apparent digestibility of Cu in citric acid supplemented groups of pigs.

5.2.8.6 Manganese Availability

The per cent availability of Mn recorded for the three treatment groups was 72.13, 76.96 and 73.16 respectively in the present study (Table 22, Fig.12). The statistical analysis of the data revealed that there was significant (P< 0.01) difference between the treatment groups. Ration T2 was showing significantly higher (P< 0.01) Mn availability than those of T1 and T3. The results indicated that phytase supplementation increased the availability of Mn in pigs fed rations without any added minerals and was better than that of the control group.

In concurrence with the present study Viswanathan *et al.* (2007) observed a better apparent digestibility of Mn in citric acid plus phytase supplemented groups.

5.2.9 Plasma Biochemical Parameters

Various plasma biochemical parameters of blood collected towards the end of the experiment from pigs maintained on the three experimental rations are shown in Table 23.

5.2.9.1 Calcium

Initial plasma Ca concentration of the animals belonging to groups T1, T2 and T3 was 10.29, 10.10 and 10.37 mg/dl respectively and the values for plasma Ca at 16th week of the experiment were 10.96, 10.56 and 10.67 mg/dl for groups T1, T2 and T3 respectively. The statistical analysis of the data on blood Ca revealed no significant ($P>0.05$) difference among pigs belonging to the treatment groups T1, T2 and T3. Pigs of all the three groups showed normal plasma Ca irrespective of phytase or Ca supplementation.

In concurrence with the present study Adeola *et al.* (1995) and Moreira *et al.* (2003) observed that plasma Ca concentration was not significantly affected by dietary phytase in pigs while Reinhart and Mahan (1986) and Qian *et al.* (1996) found that serum Ca concentrations were higher when dietary Ca:P ratio was increased.

5.2.9.2 Phosphorus

Plasma P concentration of the three treatment groups was 5.00, 5.03 and 5.01 mg/dl respectively initially, and 5.22, 5.82 and 5.46 mg/dl respectively at 16th week. On statistical analysis there was no difference ($P>0.05$) in plasma P concentration among the animals fed rations T1, T2 and T3 indicating that phytase supplementation or removal of mineral supplementation had no effect on plasma P concentration. In concurrence with the present study Moreira *et al.* (2003) observed that phytase levels did not affect plasma Ca and P or Ca:P ratio.

In contrast with the present study Cromwell *et al.* (1970) reported that by increasing the dietary P from 0.34 to 0.56 per cent to grower and from 0.29 to 0.44 per cent to finisher ration resulted in a linear increase in serum P. Lei *et al.* (1993), Han *et al.* (1997) and Liu *et al.* (1997) observed that supplemental phytase significantly enhanced plasma concentrations of iP in pigs. Martinez *et al.* (2004) and Jendza *et al.* (2005) also could observe higher plasma P concentration in pigs fed phytase supplemented diets.

But Kornegay *et al.* (1981b) observed that serum P of barrows, boars and gilts were reduced when less than NRC suggested levels of P were fed. Kornegay *et al.* (1981a) and Qian *et al.* (1996) observed that serum P was decreased when dietary Ca and P were increased which disagrees with with the results of the present study.

5.2.9.3 Magnesium

The initial plasma magnesium concentrations of the animals belonging to the three dietary treatments were 2.58, 2.89 and 3.10 mg/dl, and the values corresponding to the final blood collection were 3.29, 3.79 and 3.71 mg/dl. The statistical analysis of the data reveals that there was no significant difference (P>0.05) between the three treatment groups. The results indicated that phytase addition to the ration containing no mineral supplements had no effect on plama Mg concentration in pigs.

Kornegay *et al.* (1981a) and Qian *et al.* (1996) in concurrence with the present result observed no difference in serum Mg, when dietary Ca and P were increased whereas Adeola *et al.* (1995) found that plasma Mg concentration was increased in response to the addition of phytase to the diet.

5.2.9.4 Zinc

Plasma Zn values from the animals belonging to the three dietary treatment groups were 0.74, 0.72 and 0.76 ppm respectively initially and 0.66, 0.68 and 0.69 ppm respectively at 16th week. Statistical analysis of the data indicated that there was no significant difference (P>0.05) among pigs fed the rations T1, T2 and T3. Phytase supplementation to rations without mineral supplementation has no effect on the plasma Zn concentration in pigs.

Qian *et al.* (1996 observed that plasma Zn was not influenced by dietary Ca:tP ratio which is in accordance with the present study.

In contrast with the present study Lei *et al.* (1993) observed that either supplemental phytase or supplemental Zn increased plasma Zn concentrations in weanling pigs. Adeola *et al.* (1995) noticed that plasma Zn concentration increased when phytase was added to the diet containing no supplemental Zn, but plasma Zn concentration was not affected by phytase, when the diet was supplemented with Zn.

5.2.9.5 Copper

The initial plasma concentration of Cu recorded for the animals belonging to the three dietary groups were 1.02, 1.13 and 1.19 ppm, respectively and the final values were 1.65, 1.69 and 1.64 ppm (Table 23). On perusal of the statistical analysis results no significant effect of phytase supplementation were observed for plasma Cu concentration as there was no significant difference (P>0.05) among pigs fed the three rations T1, T2 and T3.

Pond *et al.* (1978) reported that diets severely deficient in Cu can be tolerated by the finishing pig and growing rat without drastic effect on net uptake of tissue Cu even in association with wide variations in diet Cu and Zn levels. Murry *et al.* (1997) observed no consistent effect of dietary microbial phytase for serum Cu concentrations in pigs fed supplemental phytase in agreement with the present study.

5.2.9.6 Manganese

Initial plasma concentrations of Mn of animals belonging to the three groups were 0.01, 0.01 and 0.02 ppm, respectively and the values corresponding to the 16th week collection was 0.02 ppm for all the three groups. On statistical analysis of the data there was no significant difference (P>0.05) between the three groups suggesting that supplementation of phytase to rations containing no added minerals had no effect on the plasma Mn concentration of pigs.

Pond *et al.* (1978) reported that uptake of tissue Mn was not affected by wide variation in the dietary Cu and Zn levels.

5.2.9.7 Alkaline Phosphatase

In the present study plasma alkaline phosphatase enzyme activity was 330.67, 339.67 and 314.33 U/L respectively initially and corresponding values obtained for the 16^{th} week collection were 609.83, 552.67 and 550.67 U/L respectively. Statistical analysis of the data revealed no significant difference (P>0.05) in plasma ALP activity among animals fed the three rations T1, T2 and T3 indicating that phytase supplementation to rations containing no supplemented minerals had no effect on plasma ALP activity. In concurrence with that of the present study Qian *et al.* (1996) observed that ALP activity were not influenced by Ca:tP ratio.

On contrary, Hoefer *et al.* (1960) observed increased serum ALP activity due to supplementation of Zn to control rations varying in Ca levels in pigs. Hoekstra *et al.* (1967) and Lei *et al.* (1993) fed corn-soyabean meal diet containing high Ca with added Zn and observed significantly higher activities of serum ALP than that of unsupplemented group. Boyd *et al.* (1983) also found that the plasma ALP activity increased with time and with the concentration of tP.

Han *et al.* (1997) reported a lower serum ALP activity in microbial phytase supplemented pigs than those fed control diets.

5.2.10 Slaughter Data

Photograph of left tenth rib of pigs belonging to the dietary treatments T1 and T3 are given as plates 4 and 5, respectively. The tenth rib of representative animal fed the control ration T1 was showing no gross abnormalities while that fed ration T3 (ration without any added minerals or phytase) was exhibiting beading of the rib bone indicating Ca deficiency though there was no external manifestation of any illness.

Data on dressing percentage, loin eye area, back fat thickness, bone ash, bone minerals such as Ca and P of the pigs maintained on the three dietary treatments are shown in Table 24 and are represented graphically in Fig. 12 to 19.

5.2.10.1 Dressing Percentage

The dressing percentage of pigs belonging to the three treatment groups were 73.76, 70.20 and 70.16 respectively. The statistical analysis of the data indicated that there was significant difference (P< 0.01) between pigs fed three rations T1, T2 and T3. The dressing percentage of pigs maintained under T1 was higher (P< 0.01) than that of T2 and T3, while there was no difference (P>0.05) in dressing percentage among pigs of T2 and T3. This indicates that even though the growth rate and feed efficiency were not affected, dressing percentage was lower in pigs reared on rations containing no added minerals. Supplementation of phytase did not improve the dressing percentage of pigs fed rations containing no added minerals.

In contrast to the results obtained in the present study O'Quinn *et al.* (1997) noticed that phytase supplementation increased dressing percentage and marbling in pigs. Sakthivel (2003) reported average dressing percentages of 72.28, 71.97, 72.33 for control as well as pigs supplemented with cuttle fish waste at two levels respectively and these values are in concurrence with the values of the present study. Sekhar (2003) also reported dressing percentage values of 71.7, 72.0 and 72.4 for Large White Yorkshire pigs in his experiment with rations supplemented with bakers yeast.

5.2.10.2 Loin Eye Area

In the present study the calculated loin eye area of the three treatment groups was 23.44, 23.38 18.25 cm^2 respectively and the difference was significant (P< 0.05) on statistical analysis. The loin eye area of pigs of T3 was lower (P< 0.05) than that of T1 and T2, indicating that the negative effect of T3 containing no added minerals was nullified by the addition of phytase. Cromwell *et al.* (1970) found that carcasses of barrows fed the low P level had significantly smaller *Longissimus dorsi* areas and lower yield of ham and loin than those of pigs fed the higher P levels, in agreement with the results of the present study.

Sakthivel (2003) reported similar values of loin eye area of 24.22, 23.87 and 22.4 cm^2 in his experiment in pigs fed with rations supplemented with cuttle fish waste silage at two different levels. Similarly Sekhar (2003) reported loin eye area of 22.0, 22.6 and 23.7 cm^2 in Large White Yorkshire pigs in his experiment with ration supplemented with bakers yeast and these values are also in accordance with the values obtained for pigs in the present study.

5.2.10.3 Back Fat Thickness

In the present study the back fat thickness recorded for the three dietary treatments was 3.40, 3.41 and 3.39 cm respectively. On statistical analysis of the data revealed that there was no significant differences ($P>0.05$) between the treatments.

Cromwell *et al.* (1970) found that carcasses of barrows fed the low P diet had significantly more back fat than those of pigs fed the higher P levels. Similarly Mavromichalis *et al.* (1999) reported that omitting the vitamin and trace mineral premixes had no effect on carcass back fat thickness in pigs. They also observed that diets containing varying Ca and P levels had no effect on meat quality or back fat thickness. Sakthivel (2003) reported back fat thickness of 3.32, 3.07 and 3.31 cm in pigs fed with rations containing cuttle fish waste silage at two levels. Sekhar (2003) also reported back fat thickness of 3.35, 3.28 and 3.32 cm in Large White Yorkshire pigs in his experiment with yeast these values are in concurrence with hat of the present study.

5.2.10.4 Weight of Internal Organs

The data on weight of internal organs such as heart, lungs, liver, kidney and spleen were taken and were expressed as percentage of carcass weight (Table 24). On statistical analysis there was no significant difference among the animals of the three treatments with regard to any of the organ weight studied. The weight of heart, lungs, liver, kidney and spleen expressed as percentage of carcass weight was 0.4, 1.51, 2.47, 0.49 and 0.25 respectively for T1, 0.4, 1.48, 2.55, 0.44 and 0.34 respectively for T2 and 0.4, 1.50, 2.47, 0.46 and 0.28 respectively for T3 animals.

Kies *et al.* (2005) noticed no difference between the weight of the entire gastrointestinal tract, heart and liver as a result of phytase supplementation which agrees with the results of the present study. Shelton *et al.* (2004) on the other hand noticed better liver and kidney weight as a percentage of final body weight with phytase supplementation.

5.2.10.5 Bone Ash Percentage

The percentage of bone ash of the animals belonging to the three dietary groups was 49.62, 48.66 and 43.69 respectively as shown in Table 25. On statistical analysis there was significant decrease ($P < 0.01$) in the percentage of bone ash of animals of group T3 compared to that of T1 and T2 and the difference between T1 and T2 was nonsignificant ($P > 0.05$). Pigs fed ration containing no added minerals but supplemented with phytase was showing similar percentage of bone ash as that of pigs fed the control ration (T1). The results of the study indicated that phytase supplementation to ration without supplemented minerals significantly increased the bone ash percentage to levels of those fed the control ration containing adequate minerals.

In concurrence with the present study Cromwell *et al.* (1970), Pond *et al.* (1978), Reinhart and Mahan (1986) and Cera and Mahan (1988) observed that increasing dietary P for grower as well as finisher pigs improved bone ash values linearly.

Harper and Kornegay (1996) in their study in growing–finishing pigs fed diets supplemented with Natuphos phytase reported the ash content of tenth rib as 32.7, 34.4, 36.7 and 38.7 per cent respectively which are lower than that obtained in the present study, where as Harper *et al.* (1997) observed increased tenth rib mineralization when phytase or P was added to the low P diet in pigs fed corn-soyabean meal diets which is in accordance with the results obtained in the present study.

Biehl *et al.* (1996), Murry *et al.* (1997), Radcliffe *et al.* (1998), Park *et al.*

(2003) and Omogbenigun *et al.* (2004) also observed improved bone ash content in weaned pigs supplemented with phytase preparations. Shelton *et al.* (2004) observed that adding phytase to low calcium and low aP diet, improved bone strength and ash per cent in pigs. Jendza *et al.* (2005) noticed a linear increase in bone ash of the metacarpals with *E.coli* phytase supplementation in starter, grower and finisher pigs.

Augspurger and Baker (2004), Dilger *et al.* (2004), Onyango *et al.* (2004) and Pillai *et al.* (2006) also observed increased bone ash percentage subsequent to phytase supplementation in chicks.

Brana *et al.* (2006) compared the effectiveness of two phytase enzymes (Phyzyme and Natuphos) in pigs and could find that bone ash per cent was lower in both the negative control and Natuphos added at 250 PU/kg but it increased linearly with increasing levels of dietary Phyzyme.

5.2.10.6 Bone Calcium

From the results of the present trial the bone calcium percentage was 19.45, 20.78 and 17.01 respectively for the three dietary treatments. There was no significant difference (P>0.05) between the treatment groups with regard to bone Ca per cent.

In concurrence with the present study Pintar *et al.* (2005) found that supplemental phytase increased the mineral levels in tibia. They also observed a statistically significant increase for Fe and Mg but not for Ca, P, Cd and Zn content in tibia whereas Akyurek *et al.* (2005) studied the effect of microbial phytase on growth performance and nutrient digestibility in broilers and found no significant effect on toe Ca and P contents in 21 day old broilers.

5.2.10.7 Bone Phosphorus

Percentage of bone P was 8.63, 8.81 and 8.05 respectively for the treatment groups T1, T2 and T3. On statistical analysis there was no significant difference (P>0.05) among pigs of the three treatment groups.

In concurrence with the present study Pintar *et al.* (2005) found no significant increase in P content of tibia as a result of phytase supplementation. Akyurek *et al.* (2005) also could not find any significant effect on P contents of toe on 21 day old broilers due to microbial phytase addition.

5.2.11 Radiological Examination

X-ray of femur of pigs belonging to the dietary treatments T1, T2 and T3 are presented as plates 6, 7 and 8, respectively and that of mandibles in plates 9, 10 and 11, respectively. There were no gross abnormalities of both bones of animals belonging to the three treatment groups could be detected on radiological examination though the rations T2 and T3 contained low Ca and P.

5.2.12 Histology of kidney

Plates 12, 13 and 14 shows histology of kidney samples collected from animals belonging to the dietary treatments T1, T2 and T3 respectively at the time of slaughter. It could be seen from the plates that there was no gross lesions in the kidney tissues of representative animals of groups fed with the three rations T1, T2 and T3 indicating that feeding of ration without any mineral supplement, supplemented with phytase alone was not showing any abnormalities of the kidney tissues due to the low Ca and P contents of the diet.

5.2.13 Economics of Gain

Data on cost of feed per kg body weight gain of pigs maintained on the three dietary treatments are presented in Table 26 and illustrated as Fig. 20. The cost of ingredients used for the study was as per the rate contract fixed by the College of Veterinary and Animal Sciences Mannuthy, for the year 2006-07. Phytase was supplied by Polchem Hygiene Laboratories PVT. Ltd, Pune at the rate of Rs.350 /kg. Cost of feed per kg body weight gain of pigs maintained on the three dietary treatments were Rs. 65.73, 59.90 and 62.44 respectively, the difference was significant ($P< 0.01$) on statistical analysis. Feed cost /kg gain of T1 pigs was higher than that of T2 ($P< 0.01$) and T3 ($P< 0.05$). But the difference in the cost of production between T2 and T3 were nonsignificant (P>0.05). It can be concluded that phytase supplementation of rations resulted in decreased feed cost / kg gain.

Sakthivel (2003) reported cost of feed/kg live weight gain of Rs. 35.15, 34.4, 32.25 for the three treatment groups in his experiment with cuttle fish waste silage and Sekhar (2003) reported cost of feed/kg live weight gain as Rs. 35.15, 34.4, 32.25 respectively in his experiment with bakers yeast for the three treatment groups. The higher cost of feed/ kg gain obtained in the present experiment can be attributed to increase in cost of feed ingredients used during this period.

From the results obtained in the second experiment, it was found that body weight of pigs belonging to T2 (phytase supplemented ration) was significantly higher than (P>0.05) that of T3 (ration without mineral or phytase supplementation) in fortnights 4, 6, 7, 8 and 9 and there was no significant difference between body weights of T1 and T2 (control ration and ration supplemented with phytase alone) in these fortnights. There was no difference in feed efficiency, nutrient digestibility and plasma biochemical parameters among pigs reared under the three dietary treatments. But the availability of Ca, P and Mn for T2 ration was higher than that of the control ration while the Mg availability was lower for T2 and T3 and Zn availability was lower for T3 than that of T1 ration. Dressing percentage was lower for T2 and T3 than that of control and loin eye area was lower for T3 than that of T1 and T2. There was no gross abnormality of femur and mandible on x-ray examination of pigs reared on three experimental rations. Histopathological examination of kidney samples also was normal for pigs of T1, T2 and T3. However ricketty beads were seen on ends of ribs on carcass evaluation in pigs reared under T3. The bone ash content was also lower for T3 animals than that of T1 and T2. Thus feeding ration without any mineral supplementation but with phytase showed improved utilization of nutrients and minerals than that of T3 during the period of 114 days of the experiment.

Summary

SUMMARY

Two feeding experiments were carried out using 100 weaned piglets to study the effect of dietary supplementation of different levels of calcium, phosphorus and phytase and their interactions on growth, nutrient digestibility, mineral availability and blood and bone mineral profile.

In experiment one, sixty-four weaned Large White Yorkshire x Desi piglets (32 male and 32 female) belonging to the Centre for Pig Production and Research, Mannuthy were used as experimental animals. Male piglets were castrated and all animals were dewormed before the start of the experiment. The piglets were divided into eight groups as uniformly as possible with regard to age, sex and weight. Piglets of each group were allotted randomly into four pens with two piglets in each pen. Piglets in each replicate were housed in separate pens in the same shed with facilities for feeding and watering and were maintained under identical management conditions throughout the experimental period of 88 days. Restricted feeding was followed throughout the experimental period by allowing them to consume as much as they could, within a period of one hour and the balance feed was collected and weighed after each feeding. Clean drinking water was provided *ad libitum* in all the pens throughout the experimental period.

The animals were fed with standard grower ration with 18 per cent CP and 3200 kcal of ME /kg of feed, upto 50 kg body weight and finisher ration with 16 per cent CP and 3200 kcal of ME /kg of feed, from 50 to 70 kg body weight. Eight dietary treatments with two levels of calcium (0.6 and 1 per cent) and two levels of phosphorus (0.3 and 0.6 per cent) were used with phytase (750 units /kg) and without phytase in a 2x2x2 factorial completely randomized design.

The pigs were weighed at the beginning of the experiment and later on at fortnightly intervals. Blood samples were collected at the beginning and subsequently

at the 12th week of the experiment using heparin as anticoagulant in clean dry test tubes. A digestibility trial was conducted at the end of the experiment to determine the digestibility of nutrients and percentage availability of minerals of the experimental diets by total collection method. Cost of feed per kg gain was calculated.

Average final body weight of pigs of the eight dietary treatments T1, T2, T3, T4, T5, T6, T7 and T8 was 48.80, 50.31, 49.88, 49.50, 49.44, 49.88, 48.88 and 47.92 kg, respectively. The average daily body weight gain of pigs was 388.00, 403.00, 399.50, 394.50, 393.25, 399.25, 384.00 and 375 g, respectively. Total DMI of pigs of treatments T1 to T8 were 179.22, 190.31, 186.69, 189.69, 190.16, 187.39, 184.96 and 181.72 kg, respectively. The animals of the eight dietary treatments registered feed conversion efficiency of 5.25, 5.37, 5.31, 5.47, 5.49, 5.33, 5.48 and 5.51, respectively.

From the statistical analysis of data it was observed that there were no differences ($P<0.05$) in average daily gain, DMI and feed efficiency among pigs reared on the eight dietary treatments with different Ca, P and phyatse levels. However there was significant interactions between Ca x P ($P<0.05$) and Ca x phytase ($P<0.01$) on DM intake. Data on the digestibility of nutrients revealed that level of dietary Ca and P and Ca x P x phytase interaction had significant effect on DM digestibility. Significantly lower DM digestibility was observed for rations T3 ($P<0.05$), T5 and T7 ($P<0.01$) than the others while the CP digestibility was similar for all the rations. Significant effects of dietary levels of P and interactions between Ca x P ($P<0.01$) and Ca x phytase ($P<0.05$) were found to affect EE digestibility. Rations T7 and T8 registered significantly lower ($P<0.01$) EE digestibility compared to other groups. Level of phytase and dietary P x phytase had significant effect as far as CF digestibility was concerned. No significant difference between the rations was noted for NFE digestibility.

On statistical analysis of the data on mineral availability it was seen that all phytase supplemented rations had significantly higher (P<0.01) Ca availability than the non supplemented rations. Interactions between dietary Ca x phytase (P<0.01) and dietary P x phytase (P<0.05) was found significant for Ca availability. There was no significant effect (P>0.05) for dietary Ca, P and phytase levels on P and Mg, Zn, Cu and Mn availability. But interactions between dietary levels of Ca x P and Ca x phytase had significant effect on Cu and Mn availability respectively.

Plasma biochemical parameters of blood collected on 85th day of the experiment from animals maintained on dietary treatments T1, to T8 showed 11.0, 11.5, 10.02, 11.94, 10.43, 10.99, 11.80 and 11.92 mg/dl of Ca, 5.65, 5.11, 5.17, 5.03, 5.68, 5.31, 5.06 and 6.00 mg/dl of P, 1.91, 1.95, 1.93, 1.92, 1.84, 1.83, 1.95, 1.86 mg/dl of Mg, 1.3, 0.95, 1.17, 1.18, 0.98, 1.12, 0.95 and 0.99 ppm of Zn, 1.12, 1.28, 1.47, 1.57, 1.33, 1.50, 1.53 and 1.72 ppm of Cu and 0.01 ppm of Mn, respectively. The activity of ALP was 334, 355, 298, 337, 317, 364, 325 and 357 U/l, respectively for animals of the groups T1 to T8. From the statistical analysis of the plasma biochemical parameters it was evident that phytase supplementation had significant effect (P< 0.01) on the plasma Ca levels of the animals fed the eight dietary treatments. The interaction between dietary Ca and P levels was also found significant (P< 0.01) for plasma Ca. Plasma P was significantly affected by dietary levels of both Ca and P and interaction between Ca x P, Ca x phytase and P x phytase (P<0.01). But in case of plasma Mg, it was observed that the effect of dietary Ca, P or phytase or their interactions were non-significant (P>0.05). In case of plasma Zn dietary Ca was having significant (P< 0.01) effect and significant interactions between dietary Ca x P (P<0.01), Ca x phytase (P<0.05) and P x phytase (P< 0.01) were observed. The dietary Ca, P and phytase levels did not affect plasma levels of Cu and Mn. In case of plasma ALP activity it was found that dietary Ca, P and phytase levels had significant (P<0.05) effect on the plasma ALP activity. The interactions between dietary Ca x P levels, Ca x phytase and Ca x P x phytase on plasma ALP activity were also significant.

Cost of feed per kg body weight gain of pigs maintained on the eight dietary treatments were Rs. 59.15, 60.19, 60.58, 62.77, 62.12, 60.71, 62.69 and 63.53, respectively for treatments T1 to T8.

The overall results obtained in the first experiment can be summarized as follows. Feeding piglets with rations containing different levels of Ca (0.6 and 1 per cent), P (0.3 and 0.6 per cent) and phytase (0 and 750 U/kg) did not affect growth rate, feed efficiency, DM intake, CP and NFE digestibility and P, Mg, Zn, Cu and Mn availability. Rations T3 (0.6 per cent Ca and 0.6 per cent P), T5 (1 per cent Ca and 0.3 per cent P) and T7 (1 per cent Ca 0.6 per cent P) had lower DM digestibility than rations T1, T2, T4, T6 and T8. Rations T4 and T8 had higher CF digestibility while rations T7 and T8 had lower EE digestibility than the rest of the rations. Feeding of phytase supplemented rations (T2, T4, T6 and T8) increased Ca availability and plasma Ca levels than those fed the rest of the rations. Pigs fed rations T1, T5 and T8 had higher plasma P than those of the other treatment groups. The cost of feed / kg gain was found lowest for T1.

Since the ration T1 with low Ca, low P without phytase gave similar growth and feed efficiency with lowest cost of production, this ration was taken as the control ration for the second experiment.

In the second experiment thirty six weaned Large White Yorkshire x Desi weaned piglets (18 males and 18 females) belonging to the Centre for Pig Production and Research, Mannuthy, were randomly selected and were divided into three groups, as uniformly as possible with regard to age, sex and weight. They were randomly allotted and to the three experimental treatments. They were fed with the three experimental rations, T1- Control ration containing 0.6 per cent calcium and 0.3 per cent phosphorus, T2 - Control ration without any mineral supplements and with 750 units of phytase/kg feed and T3-Control ration without phytase and mineral supplementation. A feeding trial for 114 days was conducted along with digestibility experiment to determine the digestibility of nutrients and availability of minerals. Blood samples were collected before the start of the experiment and on 85th day. Animals were weighed initially and then after every fortnight. Radiological examination of femur and mandibles of the representative animals of three groups at the end of the study were done. All the male animals were slaughtered on attaining slaughter weight of 70 kg and the data on dressing percentage, loin eye area, back fat thickness and weight of internal organs were collected. Photograph of rib bone was taken during slaughter and bone ash percentage, calcium and phosphorus contents of bone were estimated. Kidney samples were examined histologically to assess pathological changes, if any, due to the experimental rations.

The results of the study revealed an average final body weight 67.79, 69.54, and 64.75kg for pigs of the three dietary treatments T1, T2 and T3, respectively. The average ADG was 0.51, 0.53 and 0.48 kg and the total DMI was 334.04 334.09 and 322.27 kg for animals of T1, T2 and T3 respectively. The feed efficiency of the animals of the three treatments were 5.65, 5.52 and 5.79, respectively. The statistical analysis of the data on body weights revealed significant difference ($P < 0.05$) between the treatment groups. There was no significant difference ($P > 0.05$) with regard to body weight between pigs of treatments T1, T2 and T3 in fortnights 0, 1, 2, 3 and 5. Further body weight of pigs belonging to T2 (phytase supplemented ration) was significantly higher than ($P < 0.05$) that of T3 in fortnights 4, 6, 7, 8 and 9, while the difference between that of T1 and T2 were non significant in all the fortnights. There was no difference in feed efficiency among pigs reared under the three dietary treatments. The percentage digestibility of the three rations T1, T2 and T3 were 86.11, 87.92 and 87.95 for DM, 83.63, 85.31 and 84.97 for CP, 52.81, 53.89 and 53.89 for EE, 55.49, 57.41 and 55.94 for CF and 92.22, 93.55 and 93.56 for NFE, respectively. There was no difference in the digestibility of nutrients among pigs reared under the three dietary treatments. But the availability of Ca, P and Mn for T2 ration was higher than that of the control ration while the Mg availability was lower for T2 and T3 than that of T1 and Zn availability was also lower for T3 than that of T1 ration.

Plasma Ca at 16th week of the experiment was 10.96, 10.56 and 10.67 mg/dl for groups T1, T2 and T3 respectively. The statistical analysis of the data on blood Ca revealed no significant (P>0.05) difference among pigs belonging to the treatment groups T1, T2 and T3. Plasma P concentration of the animals belonging to T1, T2 and T3 were 5.22, 5.82 and 5.46 mg/dl respectively at 16th week. On statistical analysis there was no difference (P>0.05) in plasma P concentration among the animals fed rations T1, T2 and T3 indicating that phytase supplementation or removal of mineral supplementation had no effect on plasma P concentration. There was no significant difference (P>0.05) between the three treatment groups with respect to plasma Mg level indicating that phytase addition to the ration containing no mineral supplements had no effect on plasma Mg concentration in pigs. There was no significant difference (P>0.05) between the plasma Zn, Cu, Mn and ALP activity of pigs fed the rations T1, T2 and T3.

Data on carcass studies revealed that the dressing percentage was lower for T2 and T3 (P<0.01) than that of control and loin eye area was lower for T3 (P<0.05) than that of T1 and T2. There was no gross abnormality of femur and mandible on x-ray examination of pigs reared on three experimental rations. Histological examination of kidney samples also did not show any abnormalities among pigs of T1 and T2 and T3. However rickety beads were seen on ends of ribs on carcass evaluation, in pigs reared under T3. The bone ash content was also lower for animals fed ration T3 than that of T1 and T2. Thus feeding ration without any mineral supplementation showed deleterious effects on growth, mineral availability and bone ash content, but supplementation of phytase improved utilization of nutrients and minerals than that of T3 during 114 days of the experiment.

Cost of feed per kg body weight gain of pigs maintained on the three dietary treatments were Rs. 65.73, 59.90 and 62.44 respectively, the difference was significant ($P < 0.01$) on statistical analysis. Feed cost /kg gain of T1 pigs was higher than that of T2 ($P < 0.01$) and T3 ($P < 0.05$). But the difference in the cost of production between T2 and T3 were non significant ($P > 0.05$). It can be concluded that phytase supplementation of rations resulted in decreased feed cost / kg gain.

From the overall results obtained from the two experiments conducted, it can be concluded that a level of 0.6 per cent Ca and 0.3 per cent added P is sufficient to meet the mineral requirements of cross bred piglets. Rearing of pigs without any mineral supplementation, which is the common practice among pig breeders of the State, will result in reduced growth performance and in bone abnormalities. Supplementation of phytase will alleviate the negative effects of mineral deficiency to a great extent by increasing the availability of minerals that are present in the feed ingredients used in the ration. However, long-term feeding experiments are to be carried out before making recommendations.

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PHYTASE SUPPLEMENTATION ON THE AVAILABILITY OF DIFFERENT MINERALS AND THEIR INTERACTIONS IN PIGS

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ABSTRACT OF A THESIS

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ABSTRACT

Two feeding experiments were conducted using 100 weaned piglets to study the effect of dietary supplementation of calcium, phosphorus and phytase in different levels and their interactions on growth, nutrient digestibility, mineral availability and blood and bone mineral profile.

In experiment one, sixty four weaned Large White Yorkshire x Desi piglets (32 castrated male and 32 female) belonging to the Centre for Pig Production and Research, Mannuthy were used as experimental animals. All animals were dewormed before the start of the experiment. The piglets were divided into eight groups as uniformly as possible with regard to age, sex and weight. Piglets of each group were allotted randomly into four pens with two piglets in each pen. Piglets in each replicate were maintained under identical management conditions throughout the experimental period of 88 days. Restricted feeding was followed throughout the experimental period and daily feed intake was recorded. Clean drinking water was provided *ad libitum* in all the pens throughout the experimental period.

The animals were fed with standard grower ration with 18 per cent CP and 3200 kcal of ME/kg of feed up to 50 kg body weight and finisher ration with 16 per cent CP and 3200 kcal of ME /kg of feed from 50 kg body weight. Eight dietary treatments with two levels of calcium (0.6 and 1 per cent) and two levels of phosphorus (0.3 and 0.6 per cent) were used with phytase (750 units /kg) and without phytase in a 2x2x2 factorial completely randomized design.

The pigs were weighed at the beginning of the experiment and later on at fortnightly intervals. Blood samples were collected at the beginning and on 85^{th} day of the experiment and a digestibility trial was conducted at the end of the experiment to determine the digestibility of nutrients and percentage availability of minerals of the experimental diets by total collection method.

The results of the first experiment indicated that the levels of Ca, P and phytase had no effect on average daily gain, feed efficiency and DM intake among pigs reared under the eight dietary treatments, while there was significant interaction between Ca x P ($P<0.05$) and Ca x phytase ($P<0.01$) on DM intake. The level of Ca and P and Ca x P x phytase interactions were significant for DM digestibility which resulted in lower DM digestibility for rations T3, T5 and T7 than that of other rations. Dietary levels of P and interaction between Ca x P ($P<0.01$) and Ca x phytase ($P<0.05$) were significant for EE digestibility. Hence rations T7 and T8 had lower ($P<0.01$) EE digestibility than the rest of the experimental rations. Crude fibre digestibility was affected by phytase and P x phytase interaction. Calcium availability was higher ($P<0.01$) in all phytase supplemented rations. Significant interactions between dietary Ca x phytase ($P<0.01$) and dietary P x phytase ($P<0.05$) were observed for Ca availability. Interactions between dietary levels of Ca x P ($P<0.05$) and Ca x phytase ($P< 0.05$) were found significant for availability of Cu and Mn respectively.

Data on blood samples collected on 85th day revealed that phytase supplementation had significant effect ($P< 0.01$) on the plasma Ca levels of the animals fed the eight dietary treatments. The interaction between dietary Ca x P levels was also found significant ($P< 0.01$) for plasma Ca. Dietary levels of both Ca ($P< 0.01$) and P and interaction between Ca x P ($P< 0.01$), Ca x phytase and P x phytase ($P<0.01$) were found to affect plasma P significantly. In case of plasma Zn dietary Ca was having significant ($P< 0.01$) effect and the interactions between dietary Ca x P ($P<0.01$), Ca x phytase ($P<0.05$) and P x phytase ($P<0.01$) were also found significant. Dietary Ca, P and phytase levels had significant ($P<0.05$) effect on the plasma ALP activity. Cost of feed per kg body weight gain of pigs was lowest for ration T1. Ration T1 with low Ca, low P and without phytase gave similar growth and feed efficiency with lowest cost of production compared to the other rations and thus T1 was taken as the control ration for the second experiment.

The second feeding experiment was conducted for 114 days using 36 weaned Large White Yorkshire x Desi weaned piglets (18 castrated males and 18 females) belonging to the Centre for Pig Production and Research, Mannuthy and the animals were randomly allotted to the three dietary treatments, T1- Control ration containing 0.6 per cent calcium and 0.3 per cent phosphorus, T2 -Control ration without any mineral supplements and with 750 units of phytase/kg feed and T3 - Control ration without phytase and mineral supplementation. A digestibility experiment was conducted to determine the digestibility of nutrients and availability of minerals. Blood samples were collected before the start of the experiment and on 16th week. Radiological examination of femur and mandibles of the representative animals of three groups at the end of the study were done. All the male animals were slaughtered on attaining slaughter weight of 70 kg and the data on dressing percentage, loin eye area, back fat thickness, weight of internal organs were collected. Photograph of rib bone was taken during slaughter and bone ash percentage, bone calcium and bone phosphorus were estimated. Kidney samples were examined histologically to assess pathological changes, if any, due to the experimental rations.

Body weight of pigs belonging to T2 (phytase supplemented ration) was significantly higher ($P<0.05$) than that of T3 in fortnights 4, 6, 7, 8 and 9, while the difference between that of T1 and T2 were non significant in all the fortnights. There was no difference in feed efficiency and digestibility of nutrients among pigs reared under the three dietary treatments. The availability of Ca, P and Mn for T2 ration was higher than that of the control ration while the Mg availability was lower for T2 and T3 than that of T1. Zinc availability was lower for T3 than that of T1 ration. Dressing percentage was lower ($P<0.01$) for T2 and T3 than that of control and loin eye area was lower ($P<0.05$) for T3 than that of T1 and T2. There was no gross abnormality of femur and mandible on x-ray examination of pigs reared on three experimental rations. Histopathological examinations of kidney samples also were normal for pigs of T1 and T2 and T3. However ricketty beads were seen on ends of ribs on carcass evaluation, in pigs reared under T3. The bone ash content was also lower ($P<0.01$) for animals fed ration T3 than that of T1 and T2. Thus feeding ration without any mineral supplementation showed deleterious effects on growth, mineral availability and bone ash content, but supplementation of phytase improved utilization of nutrients and minerals than that of T3 during the period of 114 days of the experiment.

Feed cost /kg gain of T1 pigs was higher than that of T2 ($P < 0.01$) and T3 ($P < 0.05$). But the difference in the cost of production between T2 and T3 were non significant ($P > 0.05$). It can be concluded that phytase supplementation of rations resulted in decreased feed cost / kg gain.