# The effects of dietary levels of inorganic phosphorus, calcium and cholecalciferol on the digestibility of phytate-P by the chick

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**Male broiler chicks (1-d-old; Ross one) were given either a control diet containing recommended levels of phosphorus, calcium and cholecalciferol or experimental diets low in P and with variable levels of Ca (normal and low) and cholecalciferol (normal or high). The low-P diet with normal levels of Ca and cholecalciferol induced a hypophosphataemia and a hypercalcaemia which was reflected in reduced tibia length and weight and in reduced Ca, P and magnesium contents of tibia. The phytate digestibility remained normal while the retention of P and Ca fell significantly. The lowering of Ca alone elevated phytate digestibility and restored P and Ca retention. The hypercalcaemia and hypophosphataemia remained and tibia mineralization remained impaired. The raising of cholecalciferol alone dramatically increased phytate digestibility and the retention of Ca and P. While this remedied the hypercalcaemia, the hypophosphataemia persisted as did the diminution of tibia weight. The simultaneous lowering of dietary Ca and elevation of cholecalciferol on low-P diets restored all variables to the levels for the control diet. Circulating levels of 1,25-dihydroxycholecalciferol were significantly elevated by low-P diets, more so with high cholecalciferol intakes. However, Ca did not influence 1,25-dihydroxy-cholecalciferol levels in plasma.**

**Calcium: Phosphorus: Cholecalciferol: Phytate: Chick**

Approximately 70% of total plant phosphorus exists as phytate-P, the availability of which is determined by a variety of nutritional factors. High levels of calcium in the diet of rats (Taylor & Coleman, 1979) and poultry (Edwards & Veltmann, 1983; Ballam *et al.* 1985) decrease the availability of phytate-P by the formation of insoluble calcium phytate, thereby reducing its hydrolysis by intestinal or plant phytases and alkaline phosphatases (Taylor, 1965). McCuaig *et al.* (1972) found that while high levels of dietary magnesium and Ca reduced both intestinal phytase (*EC* 3.1.3.26) and alkaline phosphatase (*EC* 3.1.3.1) activities in the chick, high levels of dietary phosphate had no effect. In contrast, increased intestinal P absorption during P deficiency may relate to enhanced synthesis of intestinal phytase and phosphatase (Kempson *et al.* 1979; Birge & Avioli, 1981). Dietary cholecalciferol has long been known to increase phytate-P digestibility and reduce the rachitogenic nature of low-Ca, high-phytate diets (Mellanby, 1950; Steenbock & Herting, 1955). The purpose of the present study was to examine whether increased phytate digestibility on low-Ca diets would allow a reduction in the use of inorganic P while using high intakes of cholecalciferol to stimulate Ca and P absorption and to maximize phytate hydrolysis.

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Table 1. *Composition of the basal diet (g/kg)**

| | |
|---|---|
| Maize | 610 |
| Soya-bean meal | 333 |
| Dried yeast | 50 |
| Sodium chloride | 5 |
| Methionine | 1 |
| Minerals and vitamins† | 1 |

* Formulated to contain (g/kg): 195 crude protein (nitrogen × 6·26), 1·5 calcium, 5 total phosphorus (2·4 phytate-phosphorus), 12·6 MJ metabolizable energy.

† Formulated to provide final concentrations (mg/kg): 900 vitamin A, 0·4 vitamin E, 10·0 thiamin, 3·8 riboflavin, 44·8 nicotinic acid, 14·0 pantothenic acid, 4·6 vitamin $B_6$, 1456 choline, 1750 magnesium, 60 iron, 82 manganese, 15 copper, 0·04 calcium, 108 zinc.

Table 2. *Calcium, phosphorus and cholecalciferol levels (g/kg) in the control and experimental diets**

| | Control | Experimental | | | |
|---|---|---|---|---|---|
| Inorganic P... | Normal | Low | Low | Low | Low |
| Ca... | Normal | Normal | Normal | Low | Low |
| Cholecalciferol... | Normal | Normal | High | Normal | High |
| Total P | 6·9 | 5·0 | 5·0 | 5·0 | 5·0 |
| Phytate-P | 2·4 | 2·4 | 2·4 | 2·4 | 2·4 |
| Inorganic P | 4·5 | 2·6 | 2·6 | 2·6 | 2·6 |
| Ca | 10·0 | 10·0 | 10·0 | 5·0 | 5·0 |
| Cholecalciferol (mg) | 12·5 | 12·5 | 1250 | 12·5 | 1250 |

* Calcium and inorganic phosphorus added to the basal diet as oystershell and sodium dihydrogen phosphate respectively; for details of composition, see Table 1.

## MATERIALS AND METHODS

### *Experimental design*

Sixty 1-d-old male broiler strain chicks (Ross one) were completely randomized into five treatments, each in triplicate groups of four. On arrival the chicks were randomized, wing-banded, weighed and placed in an electrically heated battery. Diet and fresh water were given *ad lib.* for 42 d. The composition of the basal diet is given in Table 1. This diet was supplemented with sodium dihydrogen phosphate, oystershell and cholecalciferol to give a control diet containing recommended levels of Ca, inorganic P and cholecalciferol (National Research Council, 1977), and experimental diets, low in P and with either low or normal levels of Ca and normal or high levels of cholecalciferol (Table 2). No animal protein was used in any of the diets. Plasma was collected into heparinized syringes from the wing vein on day 26 to measure ionized Ca. Droppings were collected on days 25, 26 and 27 at 18.00 hours and frozen at −20° for subsequent mineral analysis. Seven chicks from each treatment were killed by decapitation on day 28 for plasma and tibia analysis, and five from each treatment on day 42 for further plasma and tibia analysis.

### *Tibia analysis*

The right tibia was removed from the carcass and cleaned of all soft tissue. The tibia were dried in an oven and extracted for 24 h in hot ethanol using a Soxhlet extractor. The fat-free tibia were dried at 80°, weighed and ashed at 600° for 8 h. The ashed samples were

cooled in a desiccator and weighed. Portions of ash were dissolved in concentrated nitric acid and, after suitable dilution, the levels of Ca, Zn and Mg were determined using atomic absorption spectrophotometry (Perkin-Elmer 280) at wavelengths of 423, 214 and 280 nm respectively. P was determined by the method of Hanson (1950).

*Diet and droppings analysis*

Chromic oxide (2 g/kg) was added to all diets and thoroughly mixed. Droppings were collected from each cage of four chicks on days 25, 26 and 27. The droppings tray was thoroughly cleaned at 08.00 hours before the commencement of a 10-h collection on each day. Samples from each group were dried at 90° for 24 h and ground in a Moulinex 531 grinder. Ground diet and droppings were ashed at 600° for 8 h, cooled in a desiccator and dried. Ca was determined by atomic absorption spectrophotometry as previously described, and P by the method of Hanson (1950). Chromium was determined in diet and droppings by atomic absorption spectrophotometry at 358 nm. Phytate-P was determined using the method of Oshima *et al.* (1964). The apparent availability of dietary Ca and P was determined by the use of $Cr_2O_3$ marker as described by Mueller (1956).

*Plasma analysis*

Ionized Ca was measured in plasma by the method of Luck & Scanes (1979). Plasma P was determined by the method of Goldenberg & Fernandez (1966) and plasma total Ca, Mg and Zn by atomic absorption spectrophotometry as previously described. Plasma 1,25-dihydroxycholecalciferol (1,25-$(OH)_2D_3$) was determined by a competitive-binding assay using rabbit intestinal cytosolic 1,25-$(OH)_2D_3$-binding protein as described by Duncan *et al.* (1983). These authors ascertained the specificity of this binding protein for cholecalciferol, 25-hydroxycholecalciferol (25(OH)$D_3$) and 1,25-$(OH)_2D_3$. We have extended the evaluation of this specificity to include 24,25-dihydroxycholecalciferol (24,25-$(OH)_2D_3$) and 25,26-dihydroxycholecalciferol (25,26-$(OH)_2D_3$), and shown both to be of equal or lower affinity than 25-(OH)$D_3$. Normally 25,26-$(OH)_2D_3$ exists in plasma only in trace quantities while 24,25-$(OH)_2D_3$ may exist in up to three to four times the level of 1,25-$(OH)_2D_3$. The method of Redhwi *et al.* (1982) for purifying 1,25-$(OH)_2D_3$ from plasma was modified to minimize the contamination of 1,25-$(OH)_2D_3$ with 25,26-$(OH)_2D_3$. The first three extractions from the Sep-Pak column were as described by Redhwi *et al.* (1982). However, the fourth extraction used a lower level of ethanol in dichloromethane (25 *v.* 30 ml/l). The volume of the final (ethanol–toluene) extract was also increased from 6 to 10 ml and an additional extract of 5 ml ethanol was applied to the column. The recovery of 1,25-$(OH)_2D_3$ from plasma using this modification of the method of Redhwi *et al.* (1982) was found to be $79{\cdot}7 \pm 1{\cdot}2$%, which compares very favourably with values recorded for the standard high-performance liquid chromatography on Sephadex LH-20 method of isolation (68%, Eisman *et al.* 1976; 69%, Caldas *et al.* 1978; 75%, Sedrani, 1984). Crystalline cholecalciferol, 25-(OH)$D_3$, 24,25-$(OH)_2D_3$, 1,25-$(OH)_2D_3$ and 25,26-$(OH)_2D_3$ were obtained from Hoffman–La Roche, Nutley, NJ. Tritiated 25-(OH)$D_3$ and 1,25-$(OH)_2D_3$ were purchased from Amersham Radiochemicals. Tritiated 25,26-$(OH)_2D_3$ was obtained from Professor A. D. Care, University of Leeds. Tritiated 24,25-$(OH)_2D_3$ was produced from tritiated 25-(OH)$D_3$ in a sample of quail kidney as described by Kenny (1976).

*Statistical analysis*

Results were analysed by analysis of variance, and differences between means determined following a significant F-test ($P < 0{\cdot}05$) using Duncan's (1955) method. The method of Kramer (1956) was used to compare treatments of unequal number.

## RESULTS

Low-P diets with normal levels of Ca significantly reduced final body-weight compared with all other treatments. This reduction in growth with low-P diets was not evident when the level of dietary Ca was also lowered (Table 3). This pattern was generally reflected in tibia fat-free weight and tibia length (Table 3). Tibia total ash was similarly reduced ($P < 0.01$) on low-P diets with normal levels of dietary Ca. However, at day 42, tibia ash was also influenced by dietary cholecalciferol ($P < 0.01$) such that the ameliorating effects of high cholecalciferol and low Ca intakes on tibia ash were additive. This pattern was reflected in the levels of tibia Ca, P and Mg at day 42.

No effects of diet on tibia Zn were found. In addition, the effects of high intakes of cholecalciferol on bone mineral composition at day 28 were evident only with the low-P diets which were also low in Ca.

Marked variation in phytate digestibility between the five dietary treatments was found (Table 4). Both high intakes of cholecalciferol and low intakes of Ca independently increased ($P < 0.05$) phytate-P digestibility on the low-P diets. These effects were additive in that the most favourable effect on phytate digestibility was observed with the lowest level of Ca combined with the highest level of cholecalciferol. P retention was significantly reduced ($P < 0.05$) as dietary inorganic P was reduced from 4·5 to 2·6 g/kg. This effect was negated by increasing cholecalciferol levels in the diet to 1250 $\mu$g/kg and further improvements were made by reducing Ca intakes ($P < 0.05$). Again, the effects of low Ca intake and high cholecalciferol intake, while being independent, were additive. A comparable pattern was found for Ca retention.

Diets which were low in P and which contained normal levels of Ca led to significant ($P < 0.01$) elevations in plasma ionized Ca at day 28 and in plasma total Ca at days 28 and 42. The levels of dietary cholecalciferol did not influence plasma Ca levels, total or ionized. Plasma P levels were significantly reduced ($P < 0.01$) in chicks given diets containing low levels of P, with the exception of those given diets which were also low in Ca but high in cholecalciferol. In general, the hypophosphataemia induced by low-P diets was significantly greater ($P < 0.05$) with normal-cholecalciferol diets, irrespective of Ca levels. No significant effect of diet was observed for either the Mg or Zn plasma levels.

Diets with low levels of inorganic P led to marked increases in circulating levels of 1,25-$(OH)_2D_3$ ($P < 0.001$). The increases were fivefold with normal levels of dietary cholecalciferol and ninefold at the high intakes of cholecalciferol. Dietary Ca did not influence the level of circulating 1,25-$(OH)_2D_3$.

## DISCUSSION

The complex interactions of dietary P, phytate, Ca and cholecalciferol on growth and mineral metabolism in the pig have been studied by Pointillarf *et al.* (1984, 1985). These authors observed that cholecalciferol supplementation considerably enhances Ca and P utilization by pigs fed on a phytate-P diet, but did not completely overcome the negative effects of phytate feeding on bone mineralization. In the present study the negative effects of a high-phytate, low-inorganic P diet were overcome by the independent but synergistic effect of lowering dietary Ca and simultaneously raising the intake of cholecalciferol.

The first dietary manipulation in the present study, that of lowering inorganic P from 4·5 to 2·6 g/kg, led to a predictable hypophosphataemia and a hypercalcaemia, to abnormalities in growth and skeletal development and to the marked rise in circulating levels of 1,25-$(OH)_2D_3$ previously recorded on lowering dietary P (Sommerville *et al.* 1985). To this low-P diet two other modifications were made, singly and then together. The introduction of a high level of cholecalciferol led to a marked increase in circulating levels of 1,25-$(OH)_2D_3$. This metabolite is known to stimulate the absorption of Ca and P and to

Table 3. *The effect of dietary calcium and cholecalciferol in low-phosphorus diets* on tibia mineralization in broiler chicks*

(Mean values with their standard errors)

| Inorganic P...<br>Ca...<br>Cholecalciferol... | Normal<br>Normal<br>Normal | | Low<br>Normal<br>Normal | | Low<br>Normal<br>High | | Low<br>Low<br>Normal | | Low<br>Low<br>High | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SE | Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| Final body-wt (g) | 1673[a] | 74 | 1126[b] | 104 | 1237[b] | 126 | 1570[a] | 37 | 1637[a] | 44 |
| | | | | Day 28 | | | | | | |
| Tibia (fat-free) (mg/g) | | | | | | | | | | |
| Wt (g) | 2·68[a] | 0·12 | 1·45[b] | 0·04 | 1·60[bc] | 0·06 | 1·83[c] | 0·10 | 1·91[c] | 0·11 |
| Total ash | 524[a] | 4 | 413[b] | 8 | 434[b] | 9 | 439[b] | 2 | 482[c] | 9 |
| Ca | 191[a] | 3 | 156[b] | 3 | 155[b] | 6 | 168[b] | 6 | 186[a] | 8 |
| P | 91[a] | 1 | 65[b] | 1 | 67[b] | 2 | 76[c] | 2 | 86[a] | 1 |
| Mg (μg/g) | 4893[a] | 89 | 3031[b] | 120 | 3788[c] | 70 | 4187[c] | 238 | 4919[a] | 113 |
| Zn (μg/g) | 258 | 16 | 258 | 12 | 236 | 14 | 262 | 7 | 244 | 8 |
| | | | | Day 42 | | | | | | |
| Tibia (fat-free) (mg/g) | | | | | | | | | | |
| Wt (g) | 4·45[a] | 0·3 | 3·1[b] | 0·2 | 3·1[b] | 0·3 | 3·2[bc] | 0·2 | 3·9[ac] | 0·2 |
| Total ash | 508[a] | 6 | 423[b] | 10 | 460[c] | 9 | 451[bc] | 14 | 491[a] | 4 |
| Ca | 196[a] | 3 | 155[b] | 6 | 173[c] | 2 | 169[bc] | 6 | 184[ac] | 4 |
| P | 88[a] | 1 | 63[b] | 2 | 72[c] | 3 | 72[c] | 3 | 86[a] | 2 |
| Mg (μg/g) | 4837[a] | 109 | 3124[b] | 184 | 4002[c] | 47 | 3404[b] | 241 | 4535[a] | 75 |
| Zn (μg/g) | 245 | 11 | 241 | 17 | 235 | 6 | 262 | 10 | 242 | 9 |
| Length (mm) | 91[a] | 1 | 77[b] | 12 | 79[b] | 3 | 86[a] | 1 | 89[a] | 1 |

[a,b,c] Mean values with unlike superscript letters differed significantly ($P < 0·01$).

* For details of composition, see Tables 1 and 2.

Table 4. *The effects of dietary calcium and cholecalciferol levels on dietary calcium and phosphorus availability, plasma 1,25-dihydroxycholecalciferol (1,25-$(OH)_2D_3$) and plasma mineral composition of broiler chicks on low-phosphorus diets**

(Mean values with their standard errors)

| Inorganic P... | Normal | | Low | | Low | | Low | | Low | |
|---|---|---|---|---|---|---|---|---|---|---|
| Ca... | Normal | | Normal | | Normal | | Low | | Low | |
| Cholecalciferol... | Normal | | Normal | | High | | Normal | | High | |
| | Mean | SE | Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| | | | | | Days 24–28 | | | | | |
| Dietary mineral availability (%) | | | | | | | | | | |
| Phytate-P digestibility | 50·1[a] | 1·5 | 50·5[a] | 1·0 | 58·5[b] | 1·7 | 64·5[c] | 1·1 | 76·5[d] | 1·1 |
| Total P retention | 41·5[a] | 1·0 | 32·0[b] | 1·2 | 40·5[a] | 1·5 | 47·5[c] | 3·0 | 54·0[d] | 1·6 |
| Ca retention | 43·5[a] | 1·3 | 38·0[b] | 0·6 | 45·5[a] | 1·6 | 52·5[c] | 1·7 | 64·5[d] | 2·9 |
| | | | | | Day 28 | | | | | |
| Plasma minerals (mmol/l) | | | | | | | | | | |
| Total Ca | 2·65[a] | 0·05 | 3·35[b] | 0·21 | 3·25[b] | 0·26 | 2·24[a] | 0·07 | 2·50[a] | 0·12 |
| Ionized Ca | 1·28[a] | 0·10 | 1·45[b] | 0·03 | 1·65[b] | 0·10 | 1·33[a] | 0·02 | 1·25[a] | 0·06 |
| P | 2·30[a] | 0·07 | 0·70[cb] | 0·05 | 1·08[c] | 0·02 | 1·40[d] | 0·11 | 2·10[a] | 0·03 |
| Plasma 1,25-$(OH)_2D_3$ (pg/ml) | 11·1[a] | 2·7 | 59·0[b] | 8·2 | 90·0[c] | 17·1 | 48·2[b] | 4·5 | 87·0[c] | 9·2 |
| | | | | | Day 42 | | | | | |
| Plasma minerals (mmol/l) | | | | | | | | | | |
| Ca | 2·65[a] | 0·06 | 3·3[b] | 0·17 | 3·53[b] | 0·06 | 2·58[a] | 0·04 | 2·55[a] | 0·06 |
| P | 2·26[a] | 0·19 | 0·65[b] | 0·06 | 0·90[c] | 0·16 | 1·26[c] | 0·26 | 1·94[a] | 0·19 |
| Mg | 0·79 | 0·03 | 0·90 | 0·03 | 0·92 | 0·07 | 0·74 | 0·08 | 0·76 | 0·04 |
| Zn | 35·7 | 2·08 | 28·9 | 2·78 | 30·2 | 2·4 | 31·7 | 1·4 | 33·3 | 1·7 |

[a,b,c,d] Mean values with unlike superscript letters differed significantly ($P < 0·05$).

* For details of composition, see Tables 1 and 2.

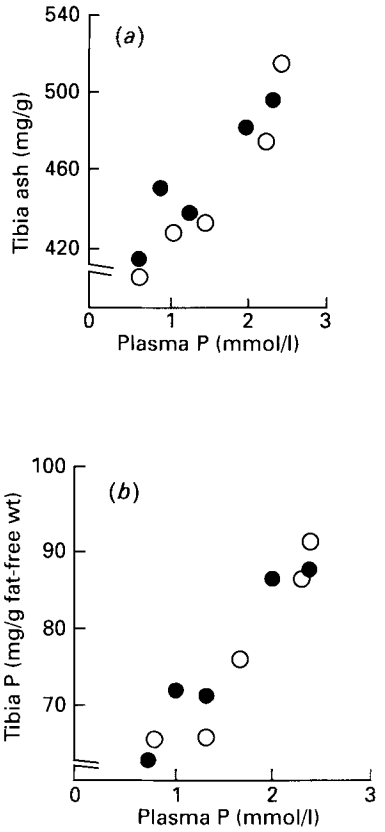


Fig. 1. The relationship between (*a*) plasma P (mmol/l) and tibia ash (mg/g) and (*b*) plasma P (mmol/l) and tibia P (mg/g fat-free weight) in broiler chicks at (○) 28 and (●) 42 d of age.

increase phytate digestibility (Mellanby, 1950; Steenbock & Herting, 1955). Such an effect was observed in the present study with significant increases in the availability of Ca and phytate and a resultant improvement in the hypophosphataemia associated with the low P intake. The second single manipulation of the basal low-P diet was to reduce Ca intake while maintaining a normal intake of cholecalciferol. This manipulation was also associated with an increased availability of phytate-P and with the retention of both P and Ca. This effect of Ca, independent of cholecalciferol has been reported previously for the chick (Vandepopulière *et al.* 1961; Harms *et al.* 1962; Ballam *et al.* 1984) and for the rat (Taylor & Coleman, 1979). Again these changes in mineral availability were associated with a significant improvement in the prevailing hypophosphataemia. In contrast to the changes seen with high intakes of cholecalciferol, the lowering of Ca led to the elimination of the low-P-induced hypercalcaemia. The marked reduction in growth observed on the basal, low-P diet was eliminated when Ca levels were reduced, but not when high levels of cholecalciferol were provided. When both these dietary modifications, lowering Ca and raising cholecalciferol, were made the adverse effects of a low-P diet were almost completely abolished, leading to a normalization of growth, of plasma mineral composition and of bone growth and composition at 42 d. These effects were achieved by a marked rise in phytate-P digestibility and in the retention of both Ca and P.

The key role played by improving P availability in reversing the effects of a low-P diet

is seen in Fig. 1 (*a* and *b*). By varying the level of cholecalciferol and Ca a graded increase in phytate digestibility leads to a graded increase in plasma P. In turn, increasing levels of plasma P led to a graded increase in tibia P and tibia ash. These results clearly show that diets containing both low levels of Ca and elevated levels of cholecalciferol permit a greater utilization of phytate-P and reduce the requirement for inorganic P. The levels of vitamin D used exceed the level normally permitted for animal feeds and may pose regulatory difficulties. However, the substantial potential cost savings on the use of endogenous plant phosphorus may merit further work in this field.

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