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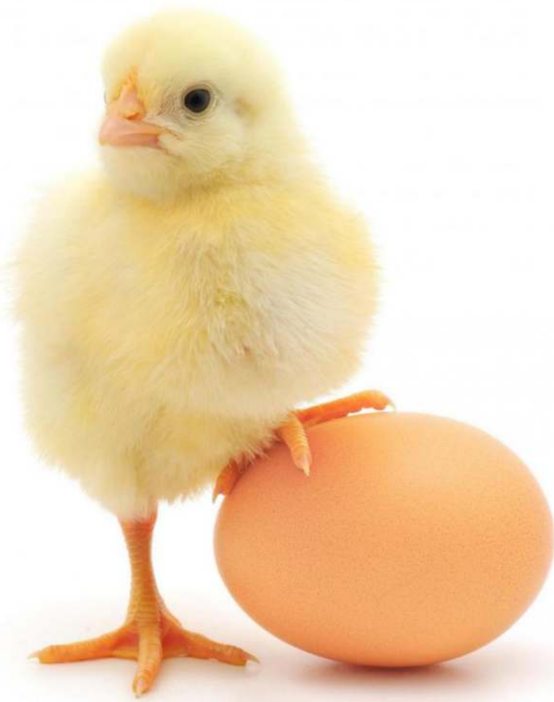

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Effects of Dietary Levels of Calcium and Nonphytate Phosphorus in Broiler Starter Diets on Live Performance, Bone Development and Growth Plate Conditions in Male Chicks Fed a Corn-Based Diet[1]

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Abstract: A study was conducted to evaluate the effects of dietary levels of calcium (Ca), nonphytate P (NPP), phytase (Phy) and 25-hydroxycholecalciferol (25-OH) on live performance and bone development in male chicks fed a corn-based diet. Dietary treatments consisted of a 2×2×4×4 factorial arrangements with two levels of supplemental phytase (0 or 1200 FTU kg^{-1}), two levels of 25-OH (0 or 69 µg kg^{-1}), four levels of Ca (0.20% less than a 2:1 ratio of Ca to NPP; 2:1 ratio of Ca to NPP; 0.20% Ca greater than a 2:1 ratio of Ca to NPP; 0.40% Ca greater than a 2:1 ratio of Ca to NPP) and four levels of NPP (0.35, 0.40, 0.45 and 0.50%) for a total of 64 treatments. The primary basal diet was supplemented with a complete vitamin mix containing 5500 IU of cholecalciferol. Each diet was fed to six replicates per treatment; each pen having 6 birds. At 18 d birds were weighed, feed consumption determined and all birds killed for bone measurements. Toes from all birds within a pen were removed and ashed. Tibiae from both legs were removed and scored for incidence and severity of tibial dyschondroplasia and for incidence of Ca or P rickets. Ca: NPP ratios and Ca levels similar or higher than NRC (1994) recommendations appear necessary for adequate bird performance. Phy supplementation improved FCR, whereas the addition of 25-OH to diets already containing 5500 IU kg^{-1} of cholecalciferol had a negative effect on FCR due to a possible hypercalcemia condition. Bone development was improved by increasing NPP and Ca levels. Moreover, supplementation with 25-OH was effective in reducing leg abnormalities. Addition of 25-OH helped to relieve leg problems when suboptimal Ca levels were supplied while Phy supplementation was effective for this purpose when high Ca levels were given. These additives could be seen as a strategy to alleviate problems with suboptimal Ca: NPP ratios.

Key words: Broilers, leg abnormalities, calcium, phosphorus, phytase and 25-hydroxycholecalciferol

Introduction

Skeletal abnormalities represent a big concern within the poultry industry as they cause considerable welfare and production problems (Venalainen *et al*., 2006; Waldenstedt, 2006). Moreover, in the American market alone it is estimated that economic losses due to broiler leg problems represent a loss of 80-million dollars (Sullivan, 1994). During the last decade increases in rate of growth in broilers has been impressive, including an increase in bone mass, which has added pressure to the machinery dedicated to accommodate the mineral requirements of the bird (Lilburn, 1994; Bar *et al*., 2003). The higher nutrient demand in a shorter period of time by the fast growing chicken used today compared to the typical chicken reared several decades ago has lead to an increased incidence of leg disorders. Havenstein *et al*. (1994) compared a fast growing strain typical of 1991 with a slow growing strain typical of 1957 and observed a significantly higher incidence of tibial dyschondroplasia (TD) in the former.

Although, it has been proposed that modern broilers have a high capacity to adapt to P or Ca deficiency (Bar *et al*., 2003; Hurwitz *et al*., 1995; Yan *et al*., 2005), it is also reported that numerous skeletal disorders are associated with dietary imbalances and deficiencies (Cook, 2000; Long *et al*., 1984a; Long *et al*., 1984b; Whitehead *et al*., 2004; Williams *et al*., 2000; Shafey, 1993). Results from Williams *et al*. (2000) illustrated the effects of various levels of Ca and nonphytate P (NPP) on predominant growth plate conditions (Fig. 1). This study suggested that feeding extremely high Ca levels, in relation to dietary NPP levels, resulted in over 80% normal growth plate conditions, while lower or intermediate levels resulted in either hypocalcemic rickets and/or a high incidence of TD. An evaluation of current industry usage levels of Ca and NPP levels[4], illustrated in Fig. 2, indicate that the typical U.S. broiler diet does not typically contain the higher levels of Ca noted by Williams *et al*. (2000), nor do they typically contain NPP levels in the higher ranges shown in Fig. 1, i.e. 0.6% or above. Due to these differences, the applicability of the data shown in Fig. 1 to commercial broiler production is questionable; therefore, this study was conducted to evaluate Ca and P levels more commonly used in the U.S. poultry industry.

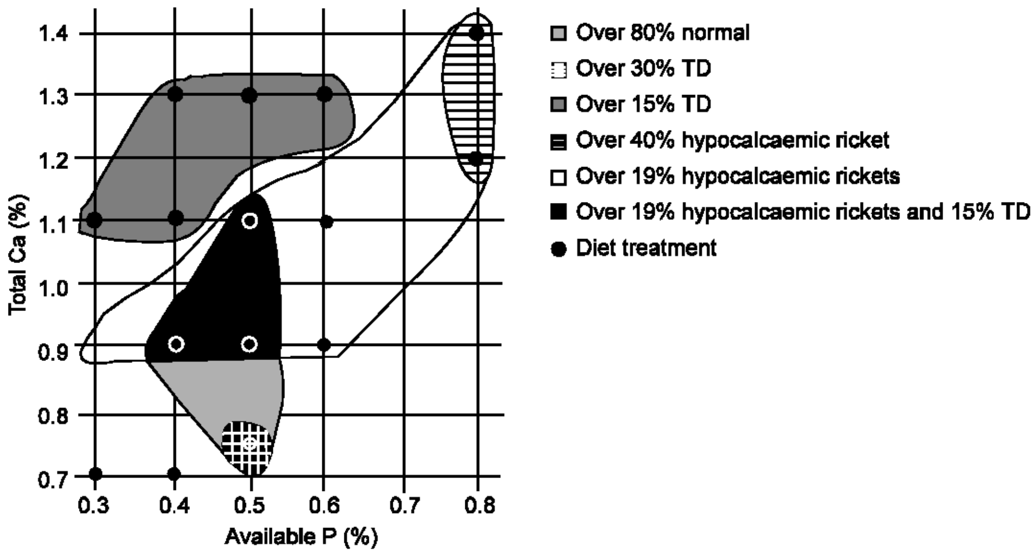

Fig. 1: Effects of various levels of Ca and NPP on predominant growth plate conditions (Adapted from Williams *et al.*, 2000)

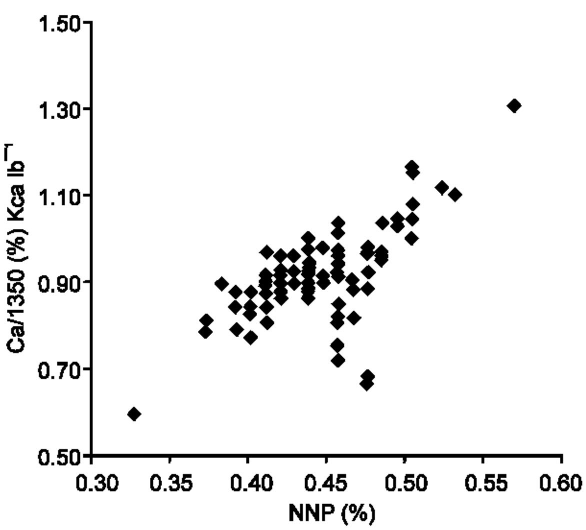

Fig. 2: Calcium and phosphorus levels from Industry Survey (Agri-Stats, Fort Wayne IN)

The use of exogenous phytase in poultry feeds is becoming more common, due in part to recent reduction in cost of phytase as well as the increasing pressure placed upon the poultry industry by environmental concerns related to land application of poultry litter (Huff *et al.*, 1998; Scheideler and Ferket, 2000; Yan *et al.*, 2001; Yan *et al.*, 2003; Summers, 1997). A commercially available form of 25-hydroxycholecalciferol (Hy-D®; DSM Nutritional Products, Parsippany NJ) has been shown in some studies to enhance bone development even when apparently sufficient levels of vitamin D_3 have been added to the diet (Angel *et al.*, 2005; Ledwaba and Roberson, 2003; Fritts and Waldroup, 2003; Zhang *et al.*, 1997; Whitehead, 1997). This study was conducted to evaluate the effect of various levels of Ca and available P on live performance and bone formation in young broilers chickens, as influenced by the addition of phytase and Hy-D.

Materials and Methods

All procedures used during this study were approved by the University of Arkansas Animal Care committee. The experimental design for this study consisted first of 2 x 2 factorial arrangement involving a basal diet using corn and soybean meal of known composition as the primary sources of energy and protein, formulated to meet current U.S. poultry industry standards (Table 1). The basal diet was supplemented with a commercial vitamin premix obtained from a local poultry integrator that provided 5500 IU kg^{-1} of cholecalciferol to the diet. Aliquots of this basal diet were then fortified with or without 1200 FTU kg^{-1} phytase supplementation (Ronozyme®, DSM Corporation, Parsippany NJ) and with or without 69 µg kg^{-1} of Hy-D for a total of four primary test diets. These four primary test diets were analyzed for Ca, total P, phytase and Hy-D activity prior to mixing the final test diets and were found to be within expected range. Within each of these four test diets, sixteen different combinations of Ca and NPP were compared. These sixteen test levels were obtained by using NPP levels ranging from 0.35-0.50%, in increments of 0.05%. While the use of 0.35% NPP in broiler starter diets is not extensive in the present industry survey, it is likely that as more producers begin using phytase and as more pressure is placed on reducing overall phosphorus excretion, greater usage may be made of this level of NPP. Within each NPP level, four calcium levels were included which are: 2:1 ratio of Ca to NPP, similar to current industry usage, 0.20% Ca below the 2:1 ratio, 0.20% Ca above the 2:1 ratio and 0.40% Ca above the 2:1 ratio. These higher Ca levels were selected to bring Ca levels into the upper ranges shown in Fig. 1.

The combination of the 16 levels of Ca and NPP within each of the four primary test diets resulted in 64 dietary treatments (Table 2). Diets were fed in a mash form. These treatments were evaluated in three consecutive trials of identical design, using the same test feed for all three trials. Two pens of six male chicks were assigned to each of the 64 test diets in each of the three consecutive trials for a total of six replicates with a total 36 birds fed each of the 64 test diets. The three experiments were considered as replications in time and were included in the statistical analysis.

Male chicks of a commercial strain (Cobb 500[5]) were obtained from a local hatchery where they had been vaccinated in ovo for Marek's disease and had received vaccinations for Newcastle Disease and Infectious Bronchitis post hatch via a coarse spray. Six chicks were placed in each of 128 compartments in electrically heated battery brooders with raised floors and given the test diets and tap water for ad libitum consumption. Fluorescent lights proved 24 h illumination. Care and management of the birds followed recommended guidelines (FASS, 1999).

The birds were placed on the test diets at day of age and grown to 18 days at which time the birds were group weighed and feed consumption was determined. The birds were killed by CO_2 inhalation. Toes were removed from all birds and ashed by pen to determine bone mineralization (Yan *et al.*, 2005). Tibiae from both legs were removed and scored by trained personnel for tibial dyschondroplasia using the scoring system of Edwards and Veltman (1983) and for incidence of calcium or phosphorus rickets (Long *et al.*, 1984a; Long *et al.*, 1984b). Any bird that died during the course of the study was weighed to adjust feed conversion.

Table 1: Composition (g kg^{-1}) and calculated analysis of basal diet

Ingredient	g kg^{-1}
Yellow corn	546.35
Soybean meal 47.5%	350.84
Alimet 10% premix	23.81
Poultry oil	17.29
Salt	5.63
Broiler premix[1]	5.00
Trace mineral mix[2]	1.00
L-Lysine HCl	1.40
L-Threonine	0.54
Pel-Stik[3]	2.50
Variable[4]	45.64
Total	1000.00
ME kcal kg^{-1}	2975.00
CP, (%) calculated	22.50
CP, (%) analyzed	21.67
Ca, (%) calculated	0.08
Ca, (%) analyzed	0.09
Total P, (%) calculated	0.37
Total P, (%) analyzed	0.42
Nonphytate P, % calculated	0.12
Met, (%)	0.57
Lys, (%)	1.33
Thr, (%)	0.91
Arg, (%)	1.49
TSAA,(%)	0.95
Sodium, % calculated	0.25
Sodium, (%) analyzed	0.25
Chloride, (%)	0.40

[1]Provides per kg of diet: vitamin A (from vitamin A acetate) 7714 IU; cholecalciferol 5511 IU; vitamin E (from dl-alpha-tocopheryl acetate) 16.53 IU; vitamin B_{12} 0.013 mg; riboflavin 6.6 mg; niacin 39 mg; pantothenic acid 10 mg; menadione (from menadione dimethylpyrimidinol) 1.5 mg; folic acid 0.9 mg; choline 1040 mg; thiamin (from thiamin mononitrate) 1.54 mg; pyridoxine (from pyridoxine HCl) 2.76 mg; d-biotin 0.066 mg; ethoxyquin 125 mg; Se 0.1 mg. [2]Provides per kg of diet: Mn (from $MnSO_4•H_2O$) 100 mg; Zn (from $ZnSO_4•7H_2O$) 100 mg; Fe (from $FeSO_4•7H_2O$) 50 mg; Cu (from $CuSO_4•5H_2O$) 10 mg; I from $Ca(IO_3)_2•H_2O$), 1 mg. [3]Pel-Stik® is a lignin sulfonate pellet binder from Uniscope Inc., Johnstown CO 80534. [4]Variable levels of ground limestone, dicalcium phosphate and washed builder's sand

Table 2: ANOVA of body weight, feed conversion, mortality, toe ash, total rickets, calcium rickets, phosphorus rickets and incidence and severity of tibial dyschondroplasia (TD)

	Probability > F							
Source of variance	BW	FCR	Mort	Ash	Ca rickets	P rickets	TD Incidence	TD severity
Nonphytate Phosphorus (NPP)	0.79	0.60	0.72	<0.0001	0.63	0.25	0.39	0.27
Calcium level (Ca)	0.05	0.03	0.52	<0.0001	0.18	0.40	<0.0001	<0.0001
Phytase (Phy)	0.81	0.06	0.01	0.01	0.07	0.08	0.32	1.00
HyD	0.45	0.03	0.49	0.34	0.09	0.26	0.12	0.81
NPP * Ca	0.83	0.07	0.32	0.43	0.34	0.65	0.21	0.54
NPP * Phy	0.66	0.96	0.04	0.59	0.93	0.49	0.19	0.21
NPP * HyD	0.28	0.52	0.41	0.73	0.36	0.27	0.14	0.13
Ca * Phy	0.85	0.99	0.25	0.14	0.54	0.60	0.91	0.98
Ca * HyD	0.29	0.88	0.72	0.92	0.68	0.83	0.16	0.68
Phy* HyD	0.11	0.36	0.04	0.16	0.07	0.43	0.59	0.34
NPP * Ca * Phy	0.99	0.94	0.50	0.97	0.13	0.94	0.66	0.53
NPP * Ca * HyD	0.99	0.69	0.62	0.93	0.25	0.98	0.43	0.65
NPP * Phy * HyD	0.98	0.07	0.76	0.18	0.01	0.92	0.83	0.88
Ca * Phy * HyD	0.22	0.74	0.06	0.92	0.23	0.90	0.85	0.68
NPP * Ca * Phy * HyD	0.33	0.53	0.84	0.05	0.64	0.72	0.73	0.98

Pens means served as the experimental unit for statistical analysis. Data were subject to analysis of variance using the general linear model procedure of SAS (1991). The model included main effects of calcium level, available phosphorus level, phytase supplementation, Hy-D supplementation and all two-way, three-way and four-way interactions. Percentage data were converted to arc sine, prior to analysis while mortality data were transformed to $\sqrt{n+1}$. Data are presented as natural numbers. Significant differences among or between means were separated by repeated *t*-tests using the least square means option of SAS software. All statements of significance are based on p = 0.05.

Results and Discussion

The ANOVA results showing the probability of various dietary factors on live performance and bone characteristics is shown in Table 2. The various factors that have a significant influence on performance will be shown in subsequent tables and figures. Non significant data will not be presented but can be obtained by contacting the authors.

The influence of the various main effects on performance and bone characteristics is shown in Table 3. Body weight was significantly affected only by the dietary calcium level. The body weight of birds fed with 0.2 % more Ca than the 2:1 Ca: NPP ratio was significantly higher than that of birds fed any other level of calcium evaluated. There was no statistical difference in body weight among birds fed diets containing 0.2 % less Ca than the 2:1 Ca:NPP ratio, the 2:1 Ca:P ratio, or 0.4% more Ca than the 2:1 Ca:NPP ratio. The 0.2% more Ca than 2:1 ratio represents dietary Ca values ranging from 0.9-1.2% Ca based on the p-levels utilized in the current study. These levels of Ca are in reasonable agreement with the 1.0% Ca suggested by NRC (1994) and suggest that up to a certain point the calcium level could increase when accompanied by increased P, reducing the risk of toxicity but more importantly improving body weight gain. This conclusion is supported by the report of Hurwitz *et al.* (1995) who found that the body weight response to dietary calcium is bell shaped and shifted to the right as the P level increased from 0.7-0.9% and with Bar *et al.* (2003) who reported a calcium requirement for optimum weight that was similar to or slightly higher than the NRC (1994) recommendation of 1.0%.

There was no significant effect of the dietary phosphorus level on body weight (Table 3), indicating that the lowest level of phosphorus used in this study was sufficient to support body weight. This in agreement with Yan *et al.* (2003); Venalainen *et al.* (2006); Yan *et al.* (2005) and Yan *et al.* (2001) but contrary to the reports of Viveros *et al.* (2002) and Sohail and Roland (1999). However, the significant responses observed by Viveros *et al.* (2002) and Sohail and Roland (1999) were obtained when diets contained lower phosphorus concentrations than used in the present study.

There was no significant effect of phytase supplementation on body weight (Table 3). Since the lowest level of dietary phosphorus was apparently adequate to support optimum body weight, it is not surprising that no significant response to phytase was observed in the present study. This in agreement with Yan *et al.* (2003) who found no significant effect of phytase supplementation on body weight. However, it is in contrast to the reports of Yan *et al.* (Yan *et al.*, 2001); Carlos and Edwards (1998); Scheideler and Ferket (2000); Viveros *et al.* (2002); Huff *et al.* (1998); Waldroup *et al.* (2000); Edwards (1993) who observed significantly higher body weight on birds fed diets containing phytase. This difference on response could be due to lower phosphorus levels evaluated in the cited studies, so that release of phosphorus would prove beneficial.

Table 3: Effects of main effects on performance of broilers

Dietary factor	18 days BW (kg)	0-18 days FCR	0-18 days Mortality	18 days Toe ash (%)	Ca rickets (%)	P rickets (%)	TD Incidence[1]	TD Severity[2]
Nonphytate P								
0.35	0.642	1.319	2.43	12.05^{b}	3.48	38.49	5.99	1.85
0.40	0.647	1.328	1.73	12.34^{b}	4.36	34.24	6.08	1.49
0.45	0.644	1.321	1.56	12.68^{a}	5.00	37.86	4.13	0.74
0.50	0.648	1.330	1.59	12.92^{a}	4.04	40.92	4.73	0.85
Calcium level								
- 0.20	0.641^{b}	1.337^{a}	2.29	12.07^{c}	5.59	36.89	10.00^{a}	2.93^{a}
2:1	0.644^{b}	1.332^{a}	2.25	12.40^{b}	4.08	38.71	6.00^{b}	1.56^{b}
+ 0.20	0.656^{a}	1.308^{b}	1.04	12.64^{ab}	4.18	35.42	3.15^{c}	0.45^{bc}
+ 0.40	0.639^{b}	1.321^{ab}	1.73	12.87^{a}	3.03	40.50	1.78^{c}	0.00^{c}
Phytase								
No	0.645	1.331^{A}	0.95^{b}	12.33^{b}	3.46	40.27	4.77	1.24
Yes	0.646	1.318^{B}	2.70^{a}	12.66^{a}	4.98	35.48	5.69	1.22
Hy-D								
No	0.647	1.317^{b}	2.08	12.44	4.93	39.03	5.97	1.29
Yes	0.643	1.332^{a}	1.58	12.55	3.52	36.73	4.49	1.18

[1]Percentage of TD scores greater than 0 (no apparent TD) based on system of Edwards and Veltmann (1983), [2]Percentage of TD scores of 3 (most severe) based on system of Edwards and Veltmann (1983), ^{abc}Means in column with same common letters do not differ significantly (p<0.05), ^{AB}Means in column with same common letters do not differ significantly (p = 0.06)

The addition of Hy-D to diets containing 5500 IU kg^{-1} of cholecalciferol gave no significant improvement in body weight, in agreement with Edwards (2002) and Roberson *et al.* (2005). Presumably, this response was due either to the level of vitamin D already supplied in the basal diet, or to the apparently adequate levels of calcium or phosphorus present in the test diets. Rao *et al.* (2006) reported a requirement of 3,182 IU kg^{-1} of cholecalciferol for optimum body weight when suboptimal amounts of Ca and P were fed. Whitehead *et al.* (2004) found that at suboptimal concentrations or ratios of Ca and NPP a vitamin D level higher than 5000 IU kg^{-1} had no positive effect on body weight. There were no significant interactions among or between the various dietary factors on body weight.

The influence of various main effects on feed conversion (FCR) is shown in Table 3. The dietary Ca level had a significant influence on the FCR. Birds fed diets with 0.2% more Ca than the 2:1 Ca:NPP had significantly better FCR than birds fed the 2:1 Ca:NPP ratio or with 0.2 % less Ca than the 2:1 Ca:P ratio. The FCR of birds fed diets with 0.4 % more Ca than the 2:1 Ca:P ratio level did not differ significantly from that of birds fed any of the other Ca levels tested. This finding suggests the need for a Ca level higher than the current NRC (1994) recommendation for optimum FCR.

There was no significant effect of dietary phosphorus level on FCR, indicating that the levels used in this study were adequate to support feed conversion (Table 3). This result is in agreement with Yan *et al.* (2003); Venalainen *et al.* (2006) and Waldroup *et al.* (2000). The latter authors reported that P levels required to sustain an adequate FCR are lower than the required to maximize body weight or tibia ash, which is also confirmed by Sohail and Roland (1999).

Phytase supplementation numerically ($p = 0.06$) improved feed conversion in the present study (Table 3). Yan *et al.* (2003) reported no effect of phytase supplementation on FCR but significant improvements have been reported by Yan *et al.* (2001); Scheideler and Ferket (2000) and Waldroup *et al.* (2000). The response to phytase may depend heavily upon the available phosphorus status of the diet, with diets having adequate levels of available phosphorus showing little or no response in feed conversion to phytase supplementation.

The addition of Hy-D to diets containing 5500 UI Kg^{-1} of cholecalciferol resulted in a significant adverse effect on feed conversion in this study (Table 3). A similar effect was reported by Fritts and Waldroup (2003) when comparing Hy-D to cholecalciferol as the vitamin D source and by Edwards (2002) when feeding high levels of vitamin D. On the other hand, Roberson *et al.* (2005) observed no effect of Hy-D on FCR which, according to the author, is typical when adequate vitamin D is supplied. The adverse response on FCR observed

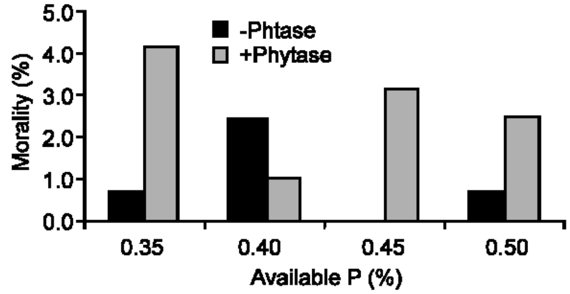

Fig. 3: Interaction of phytase supplementation and available phosphorus levels on 0-18 d mortality in male broiler chicks

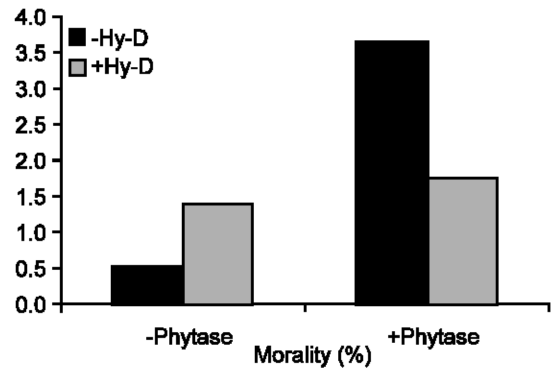

Fig. 4: Interaction between phytase and Hy-D © on 0-18 d mortality in male broiler chicks

when Hy-D was added could possibly be due to a hypercalcemic condition brought about by increased Ca absorption. There were no significant interactions among or between the different variables for feed conversion in this study.

The influence of various main effects on mortality is shown in Table 3. There were no significant effects of available P, calcium levels, or Hy-D supplementation on mortality. Birds fed diets containing phytase showed significantly higher mortality than birds fed diets without the enzyme. This in contrast to previous studies from our laboratory that reported no adverse effects of phytase on mortality (Waldroup *et al.*, 2000; Yan *et al.*, 2003) and by Sohail and Roland (1999) who observed lower mortality when phytase was added to low phosphorus diets.

There was a significant interaction between phytase supplementation and available phosphorus level for mortality (Fig. 3). Addition of phytase resulted in higher mortality when the diet contained 0.35, 0.45 and 0.50% available P but not when the diet contained 0.40% available P. A significant interaction was also found between Hy-D and phytase for mortality (Fig. 4). Birds fed diets containing phytase alone had a significantly higher mortality than the birds receiving no phytase with or without Hy-D. Mortality data are subject to extreme variability, especially when dealing with small number of animals as in the present study. Thus, the adverse effect noted when phytase was added to the diets should not be given a great deal of emphasis, since, the majority of reports dealing with phytase indicate no adverse effects or even reduction in mortality.

The influence of various main effects on toe ash is shown in Table 3. Toe ash was significantly influenced by available phosphorus, calcium level and phytase supplementation. Toe ash increased as the dietary phosphorus level increased, with the toe ash of birds fed 0.45% NPP and 0.50% available phosphorus being significantly greater than that of birds fed with 0.35 or 0.40%. This in agreement with Yan *et al.* (2001); Viveros *et al.* (2002); Yan *et al.* (2005); Venalainen *et al.* (2006) and Yan *et al.* (2003). Toe ash also increased with increasing dietary calcium levels, with the toe ash of birds fed the diet with 0.4% Ca greater than the 2:1 ratio being significantly greater than that of birds fed the diet with 0.2% less than the 2:1 ratio or birds fed the 2:1 ratio. These results suggest that dietary calcium levels equal or greater than those suggested by NRC (1994) are needed for optimum bone mineralization. This finding also confirms the reports of Bar *et al.* (2003) and Whitehead *et al.* (2004) who reported that the Ca need for bone ash is higher than the NRC recommendation. Toe ash was also significantly improved by phytase supplementation of the diets, in agreement with Yan *et al.* (2001; 2003); Edwards (1993); Carlos and Edwards (1998); Catala *et al.* (2006) and Viveros *et al.* (2002). It is in contrast to the findings of Scheideler and Ferket (2000); Sohail and Roland (1999) and Waldroup *et al.* (2000) who explained this difference in response by the fact that phytase supplementation at high NPP levels does not offer a significant contribution to increase bone mineralization.

Toe ash was not significantly affected by Hy-D supplementation, in agreement with Roberson *et al.* (2005) and contrary to the reports of Edwards (2002) and Fritts and Waldroup (2003). It is probable that the 5500 ICU kg^{-1} of vitamin D supplied within the basal diet and the combination of Ca and P levels used in the study reduced the possible influence of Hy-D, as compared to the response observed by Rao *et al.* (2006) and Baker *et al.* (1998) where suboptimal Ca and P levels or ratios were fed.

There were no significant two-way or three-way interactions among the main effects for toe ash. There was a significant four-way interaction among available phosphorus, calcium level, phytase and Hy-D supplementation but this followed no clear-cut pattern.

There were no significant main effects on the incidence of calcium rickets or phosphorus rickets. There was an interaction among available phosphorus, phytase and Hy-D supplementation on incidence of calcium rickets (Table 4) but this seemed to follow no clearcut pattern. No interactions were noted among or between the main effects for incidence of phosphorus rickets.

Table 4: Effects of three-way interaction among nonphytate phosphorus, phytase and Hy-D on percent of calcium rickets at 18 days

		Available P (%)			
Phytase	Hy-D	0.35	0.40	0.45	0.50
No	No	2.75^{bcd}	4.92^{abcd}	7.08^{ab}	4.88^{abcd}
	Yes	2.21^{cd}	1.71^{d}	1.71^{d}	2.42^{cd}
Yes	No	7.92^{a}	3.63^{abcd}	4.46^{abcd}	3.79^{abcd}
	Yes	1.04^{d}	7.21^{ab}	6.75^{abc}	5.08^{abcd}

^{abcd}Means with common letters do not differ significantly ($p<0.05$)

Increasing dietary calcium levels significantly reduced both the incidence and severity of tibial dyschondroplasia (Table 3). Birds fed diets with 0.20 or 0.40% more Ca than the 2:1 Ca:NPP ratio had significantly lower incidence of TD than birds fed diets with 0.20 less Ca or those fed the 2:1 Ca:NPP ratio. The severity of TD was also reduced when the dietary Ca level increased, with the incidence of TD among birds fed diets with 0.40 % more Ca than the 2:1 Ca:NPP ratio being significantly lower than those fed the diets with the 2:1 Ca:NPP ratio or lower. This in agreement with the report of Whitehead *et al.* (2004) who observed higher TD incidence in diets with low calcium levels.

Supplementing diets with phytase had no significant effect on any of the factors but resulted in numerical increase ($p = 0.07$) in the incidence of calcium rickets and a numerical decrease ($p = 0.08$) in the incidence of phosphorus rickets. Supplementing diets with Hy-D had no significant effect on any of the factors but resulted in numerical decreases in total rickets ($p = 0.12$), calcium rickets ($p = 0.09$) and incidence of tibial dyschondroplasia ($p = 0.12$). Fritts and Waldroup (2003) and Zhang *et al.* (1997) observed lower TD incidence when $25(OH)D_3$ was added to the diet. However, Roberson *et al.* (2005) observed no effect of Hy-D on TD incidence. The high level of cholecalciferol in the basal diets in the present study may have lessened any probable impact of Hy-D addition to the diets, as Fritts and Waldroup (2003) noted that Hy-D tended to be more effective than cholecalciferol in diets with low levels of vitamin D supplementation with little or no difference when diets contained higher levels of vitamin D.

Conclusion: Ca:NPP ratios and calcium levels similar or even higher than the recommended by the NRC (1994) are necessary for adequate bird performance. Phytase supplementation improved FCR, whereas the Hy-D addition to diets containing an adequate vitamin D level caused a negative effect on FCR due to a probable Hypercalcemia condition. Bone development was improved by increasing phosphorus and calcium levels. Consistent was the reduction of leg abnormalities by increasing Ca levels. Hy-D addition helped to relieve leg problems when suboptimal calcium levels were supplied while the phytase supplementation was effective for this purpose when high Ca levels were given. The addition of these additives could be seen as an strategy to alleviate suboptimal Ca:NPP ratios.

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