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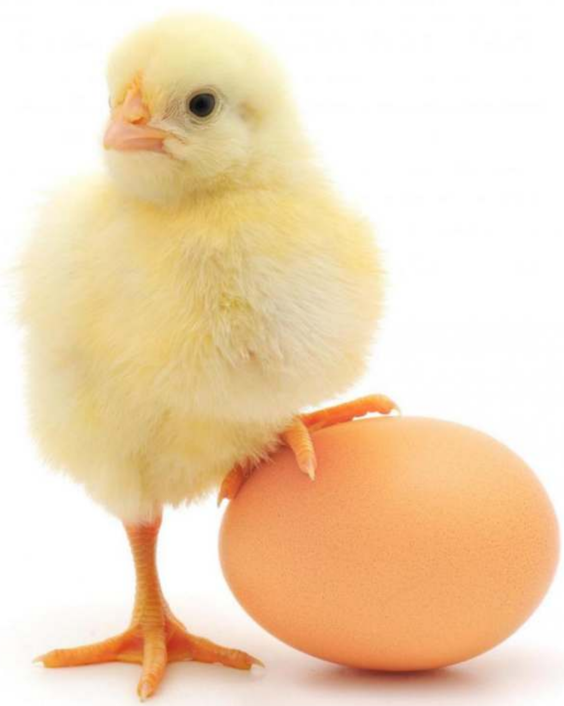

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Effects of Dietary Levels of Calcium and Nonphytate Phosphorus in Broiler Starter Diets on Live Performance, Bone Development and Growth Plate Conditions in Male Chicks Fed a Wheat Based Diet[1]

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Abstract: A study was conducted to evaluate the effects of dietary levels of calcium, nonphytate P (NPP), phytase and 25-hydroxycholecalciferol (25-OH) on live performance and bone development in male chicks fed a wheat-based diet. Dietary treatments consisted of a 2 x 2 x 4 x 4 factorial arrangements with two levels of supplemental phytase (0 or 1200 FTU/kg), two levels of 25-OH (0 or 69 µg/kg), four levels of calcium (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 used to mix all experimental diets was supplemented with a complete vitamin mix containing 5500 IU of cholecalciferol. Each diet was fed to two pens of six male chicks of a commercial broiler strain in electrically heated battery brooders for three consecutive trials using the same diet mix for a total of six replicates per treatment. 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 calcium or phosphorus rickets. Ca: NPP ratios and calcium levels similar or higher than NRC (1994) recommendations appear necessary for adequate bird performance. Phytase supplementation improved performance parameters such as FCR and body weight, whereas the addition of 25-OH to diets already containing 5500 IU/kg of cholecalciferol had a negative effect on FCR due to a possible hypercalcemia condition. Bone development was improved by increasing phosphorus and calcium levels. Moreover, supplementation with 25-OH and its combination with phytase were effective in enhancing bone development. Increasing Ca levels consistently reduced leg abnormalities. Addition of 25-OH helped to relieve leg problems when suboptimal calcium levels were supplied while 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 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 to 120 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 (Hurwitz *et al.*, 1995; Bar *et al.*, 2003; 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 on predominant growth plate conditions (Fig. 1). This study suggested that feeding extremely high Ca levels, in relation to dietary P levels, resulted in over 80% normal growth plate conditions, while more 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

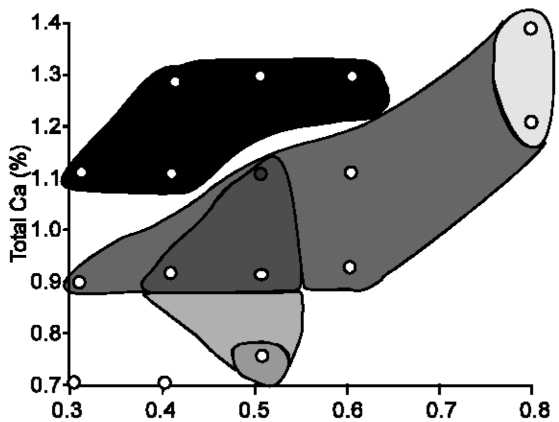

Fig. 1: Effects of various levels of Ca and available P on predominant growth plate conditions (Adapted from Williams *et al.* (2000)).

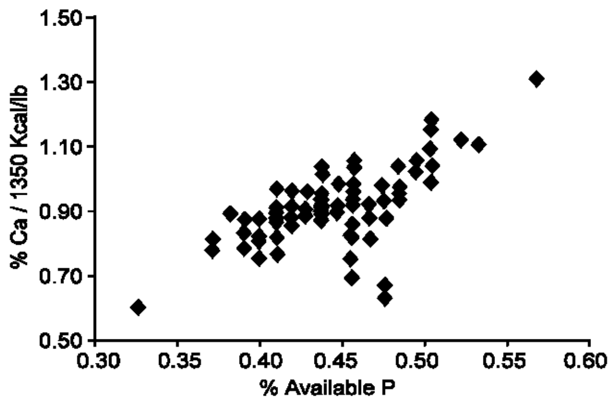

Fig. 2: Current industry usage levels of Ca and P levels (Survey by Agri Stats, Fort Wayne IN).

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. The applicability of the data shown in Fig. 1 to commercial broiler production is questionable due to the higher Ca and P levels used in the study; therefore this study was conducted to evaluate Ca and P levels more commonly used in the U.S. poultry industry.

Wheat is the primary cereal grain in most of the European countries as well as Australia and is sporadically used as a portion of the cereal grain in the United States. Wheat contains some natural phytase activity that might influence calcium and phosphorus absorption in young chicks (Anderson, 1915; Sebastian *et al.*, 1998; Vohra and Satyanarayana, 2003). 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; Fritts and Waldroup, 2003; Ledwaba and Roberson, 2003; Zhang *et al.*, 1997; Whitehead, 1997). This study was conducted to evaluate the effect of various levels of Ca and Nonphytate P on live performance and bone formation in young broilers chickens fed wheat-based diets, 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 wheat basal diet formulated to meet current U.S. poultry industry standards (Table 1) with or without 1200 FTU/kg phytase supplementation (Ronozyme®, DSM Corporation, Parsippany NJ) and plus or minus 69 µg/kg of Hy-D for a total of four primary test diets. The basal diet was supplemented with a commercial vitamin premix that provided 5500 IU/kg of cholecalciferol to the diet. These four primary test diets were analyzed for Ca, total P, phytase activity and content of 25-OH cholecalciferol prior to mixing the final test diets and were found to be within expected range for the respective nutrients. Within each of these four test diets, sixteen different combinations of Ca and NPP were compared. These sixteen test levels were selected by using NPP levels ranging from 0.35 to 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 data were included which are: 1) 2:1 ratio of Ca to NPP; 2) 0.20% Ca below the 2:1 ratio; 3) 0.20% Ca above the 2:1 ratio and 4) 0.40% Ca above the 2:1 ratio. These higher Ca levels are added 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 a total of 64 dietary treatments. Diets were fed in a mash form. These diets were evaluated in three consecutive trials of identical design, using the same test feed for all three

Table 1: Composition (g/kg) and calculated analysis of experimental diets

Ingredient	g/kg
Wheat	552.37
Soybean meal 47.5%	319.48
Alimet 10% premix	29.58
Poultry oil	36.23
Salt	5.10
Broiler premix[1]	5.00
Trace mineral mix[2]	1.00
L-Lysine HCl	2.30
L-Threonine	1.33
Pel-Stik[3]	2.50
Variable[4]	45.11
TOTAL	1000.00
ME kcal/kg	2975.00
CP, % calculated	22.50
CP, % analyzed	23.91
Ca, % calculated	0.09
Ca, % analyzed	0.17
Total P, % calculated	0.38
Total P, % analyzed	0.44
Nonphytate P, % calculated	0.13
Met, %	0.59
Lys, %	1.33
Thr, %	0.91
Arg, %	1.39
TSAA, %	0.95
Sodium, % calculated	0.25
Sodium, % analyzed	0.21
Chloride, %	0.40

[1]Provides per kg of diet: vitamin A (from vitamin A acetate) 7714 IU; cholecalciferol 5500 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 MnSO4·H20) 100 mg; Zn (from $ZnSO_4 \cdot 7H_2O$) 100 mg; Fe (from $FeSO_4 \cdot 7H_2O$) 50 mg; Cu (from $CuSO_4 \cdot 5H_2O$) 10 mg; I from $Ca(IO_3)_2 \cdot H_2O$), 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.

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 for 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 compartment in electrically heated battery brooders with raised floors and given the test diets and tap water for ad libitum consumption. Fluorescent lights proved 24 hr 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 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 for tibial dyschondroplasia using the scoring system of Edwards and Veltmann (1983) and for incidence of calcium or phosphorus rickets (Long *et al.,* 1984a; 1984b) by a group of trained evaluators. Any bird that died during the course of the study was weighed to adjust feed conversion.

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, nonphytate 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 $P \leq 0.05$ unless otherwise stated.

Results and Discussion

Dietary effects on body weight, feed conversion, mortality and toe ash: The ANOVA Table detailing the probability of various dietary factors on body weight, feed conversion, mortality and toe ash is shown in Table 2. The various factors that have a significant effect on performance are shown on subsequent Tables. Non-significant data will not be presented but can be obtained by contacting the authors.

The influence of the various main effects on body weight is shown in Table 3. Body weight was significantly influenced by dietary calcium level and by phytase supplementation. Birds fed 0.2 % less Ca than the 2:1 Ca: NPP ratio had a significant lower body weight than birds fed with the 2:1 Ca: NPP ratio and higher Ca levels evaluated in this experiment. The 0.2 % less Ca than the 2:1 Ca: NPP ratio represents nominal Ca values from 0.5% to 0.8% according to the different phosphorus levels evaluated; therefore, this result suggests that the calcium level recommended by the NRC (1994) is adequate to support body weight and affirms the results of Bar *et al.* (2003). Phytase supplementation resulted in a significant improvement on body weight, in agreement with Yan *et al.* (2001), Carlos and Edwards (1998), Scheideler and Ferket (2000), Viveros *et al.* (2002), Huff *et al.*(1998), Waldroup *et al.* (2000) and Edwards (1993). There was only one significant two-way interaction among the various factors for body weight (Table 4). A significant interaction occurred between the NPP and the

Table 2: ANOVA of body weight, feed conversion, mortality, and toe ash

	BW		FCR		Mortality		Ash	
Source of variance	Prob>F	SEM	Prob>F	SEM	Prob>F	SEM	Prob>F	SEM
NonphytateP (NPP)	0.7200	0.005	0.080	0.009	0.870	0.550	<.0001	0.109
Calcium level (Ca)	0.0100	0.005	0.001	0.009	0.730	0.553	<.0001	0.109
NPP * Ca	0.0400	0.011	0.670	0.017	0.730	1.120	0.9200	0.226
Phytase (Phy)	<.0001	0.003	0.010	0.006	0.850	0.388	0.3300	0.077
NPP * Phy	0.4500	0.008	0.740	0.012	0.720	0.783	0.4500	0.156
Ca * Phy	0.9300	0.008	0.440	0.012	0.680	0.783	0.8400	0.156
NPP * Ca * Phy	0.9200	0.015	0.940	0.024	0.370	1.622	0.8800	0.319
HyD	0.1200	0.003	0.010	0.006	0.410	0.388	0.0100	0.077
NPP * HyD	0.5400	0.008	0.260	0.012	0.160	0.783	0.0900	0.154
Ca * HyD	0.2200	0.008	0.340	0.012	0.590	0.783	0.3600	0.154
NPP * Ca * HyD	0.8700	0.015	0.750	0.024	0.670	1.622	0.4700	0.319
Phy* HyD	0.5000	0.005	0.480	0.009	0.230	0.550	0.0010	0.108
NPP * Phy * HyD	0.5700	0.011	0.630	0.018	0.440	1.120	0.5800	0.221
Ca * Phy * HyD	0.9600	0.011	0.140	0.018	0.710	1.093	0.9400	0.215
NPP * Ca * Phy * HyD	0.500	0.020	0.690	0.038	0.120	2.1870	0.7300	0.472
CV	7.820		6.390		309.220		8.3700	

Table 3: Effects of main effects on body weight, feed conversion, mortality, and toe ash of broilers

Dietary factor		18 d BW (Kg)	0-18 d Feed: Gain FCR	0-18 d Mortality (%)	18 d Toeash (%)
% Nonphytate P					
	0.35	0.655	1.341[A]	2.083	12.017[c]
	0.40	0.655	1.336[AB]	1.771	12.601[b]
	0.45	0.658	1.315[B]	1.563	12.720[b]
	0.50	0.662	1.317[B]	1.476	13.080[a]
Calcium level					
	- 0.20	0.642[b]	1.359[a]	2.031	12.163[b]
	2:1	0.666[a]	1.323[b]	1.910	12.653[a]
	+0.20	0.662[a]	1.312[b]	1.736	12.783[a]
	+0.40	0.60[a]	1.315[b]	1.215	12.820[a]
Phytase supplementation					
	No	0.647[b]	1.339[a]	1.667	12.552
	Yes	0.668[a]	1.316[b]	1.780	12.657
Hy-D supplementation					
	No	0.653	1.316[b]	1.493	12.467[b]
	Yes	0.662	1.338[a]	1.953	12.742[a]

[ab]Means with common superscripts do not differ significantly ($P < 0.05$).
[AB]Means with common superscripts do not differ significantly ($P < 0.10$).

Ca level. At 0.35 % NPP, the birds fed 0.2 % less Ca than the 2:1 ratio had significantly lower body weight than did those fed the 2:1 ratio or 0.2% more than the 2:1 ratio. However, when the Ca level was increased further to 0.4% NPP birds fed the diets with 0.2% less Ca than the 2:1 Ca: NPP ratio level had significantly lower body weight than those fed the remaining calcium levels. At NPP levels of 0.45 % NPP and 0.50 %, NPP there was no significant influence of dietary calcium levels on body weight. This indicates that Ca: NPP ratio itself is not the sole criteria for calcium adequacy; rather, total Ca itself is also important to consider. There were no significant three-way interactions among phosphorus, calcium, phytase supplementation and the addition of Hy-D on body weight.

The influence of various main effects on feed conversion rate (FCR) is shown in Table 3. There was a numerical effect of dietary phosphorus level on FCR (P=0.08) in which FCR was highest when the two lowest NPP levels were fed. This result is not in agreement with the findings of Yan *et al.* (2003), Yan *et al.* (2001), Venalainen *et al.* (2006) and Waldroup *et al.* (2000). Waldroup *et al.*(2000) utilized a P level as low as 0.20% NPP level with no significant difference on FCR. Waldroup *et al.* (2000) and Sohail and Roland (1999) stated that an adequate FCR could be obtained with less P level than the required to maximize body weight or tibia ash.

The FCR was significantly influenced by the dietary Ca level. Birds fed with 0.2% less Ca than the 2:1 Ca: NPP had significantly higher FCR compared to the birds fed the remaining Ca levels evaluated in the present study. This result indicates that calcium levels below the NRC (1994) recommendation are suboptimal for supporting feed conversion.

Phytase supplementation had a positive effect on FCR, which was significantly improved when birds were fed diets containing the enzyme, in agreement with Yan *et al.* (2001), Scheideler and Ferket (2000) and Waldroup (2000). Birds fed diets in which Hy-D was added to a diet containing 5500 UI/Kg of cholecalciferol had significantly worse FCR compared to those birds receiving diets containing without Hy-D added. Similar results have been reported by Fritts and Waldroup (2003) when comparing Hy-D as a source of 25-hydroxycholecalciferol 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 present in the diet. Considering the calcium levels supplied and the fact that Hy-D overcomes the rate limiting step where cholecalciferol is converted to 25 $(OH)_2D_3$ (Edwards, 2002), the adverse FCR observed could be due to an hypercalcemia condition. There were no significant two-way or three-way interactions among nonphytate phosphorus, calcium level, phytase supplementation and Hy-D supplementation on feed conversion.

The influence of various main effects on mortality is shown in Table 4. There were no significant main effects of nonphytate phosphorus, calcium level, phytase, or Hy-D supplementation on mortality, nor were there any significant two-way or three-way interactions among nonphytate phosphorus, calcium level, phytase supplementation and Hy-D supplementation on mortality.

The influence of various main effects on toe ash is shown in Table 3. Toe ash was significantly influenced by nonphytate phosphorus, calcium level and Hy-D supplementation, but was not significantly affected by phytase supplementation. This finding is in contrast to the reports of Yan *et al.* (2003), Edwards (1993), Yan *et al.* (2001), Carlos and Edwards (1998), Catala *et al.* (2006) and Viveros *et al.* (2002). However, it is supported by the reports of Scheideler and Ferket (2000), Sohail and Roland (1999) and Waldroup *et al.* (2000) who justified this lack of effect of the enzyme on the presence of high NPP levels in the diet, minimizing the response to the phosphorus released from the phytate molecule. When the nonphytate phosphorus level was increased, the toe ash concentration was significantly increased as well, with birds receiving the 0.50 % NPP level having the highest toe ash concentration. This in agreement with the results of Yan *et al.* (2003), Yan *et al.* (2001), Viveros *et al.*(2002), Yan *et al.* (2005) and Venalainen *et al.* (2006).

Table 4: Interaction between nonphytate phosphorus and calcium level on 18 d Body weight (kg)

	% Nonphytate P			
Calcium	0.35	0.40	0.45	0.50
- 0.20%	0.628^{de}	0.620^{e}	0.652^{abcd}	0.667^{abc}
2:1	0.677a	0.671^{abc}	0.655^{abcd}	0.660^{abc}
+ 0.20	0.668^{abc}	0.656^{abcd}	0.665^{abc}	0.660^{abc}
+ 0.40	0.645^{bcde}	0.672^{ab}	0.659^{abc}	0.662^{abc}

^{abcde}Means with common superscripts do not differ significantly (P<0.05).

Table 5: Interaction between phytase and Hy-D on 18 d percent toe ash

	Added Phytase	
	No	Yes
No Hy-D	12.594^{b}	12.340^{b}
Plus Hy-D	12.510^{b}	12.975^{a}

^{ab}Means with common superscripts do not differ significantly (P<0.05).

There was also a significant effect of dietary calcium level on toe ash. Birds fed diets containing 0.2 % less Ca than the 2:1 Ca: NPP ratio, which represented Ca values ranging from 0.50% to 0.80%, had a significantly lower toe ash concentration compared to those fed the higher Ca levels. This result is supported by the results of Bar *et al.* (2003) and Whitehead *et al.* (2004). The latter observed lower tibia ash and a higher vitamin D requirement when feeding 0.8% Ca in conjunction with 0.35% NPP, suggesting that this Ca level is suboptimal; moreover, the same author concluded that more than the 1.0% Ca recommended by the NRC (1994) is required for better bone formation. The addition of Hy-D also resulted in a significant improvement in toe ash concentration, even when the diet already contained 5500 UI/kg cholecalciferol. This is in agreement with Fritts and Waldroup (2003) and Edwards (2002) who observed a significant increase in toe ash when adding 25-hydroxycholecalciferol. On the other hand, this result is contrary to that observed by Roberson *et al.* (2005).

There was only one significant two-way interaction on toe ash, with a significant interaction between phytase supplementation and Hy-D addition (Table 5). Birds fed diets containing phytase and Hy-D had higher toe ash concentration than those birds fed diets containing with phytase alone or when Hy-D was added alone. This suggests that by adding both phytase and Hy-D the availability of phosphorus and calcium is increased in a

Table 6: ANOVA of Tibial dyschondroplasia (TD) and rickets scores

	Total Rickets		Ca Rickets		P Rickets		TD Incidence		TD Severity	
Source of variance	P > F	SEM	P > F	SEM	P > F	SEM	P > F	SEM	P > F	SEM
Nonphytate P (NPP)	0.38	2.683	0.7700	0.968	0.42	2.622	0.6000	0.936	0.240	0.446
Calcium level (Ca)	0.94	2.699	<.0001	0.974	0.69	2.639	<.0001	0.936	0.005	0.446
NPP * Ca	0.52	5.365	0.8300	1.936	0.47	5.245	0.0900	1.860	0.470	0.887
Phytase (Phy)	0.13	1.909	0.9100	0.689	0.13	1.866	0.4500	0.662	0.700	0.315
NPP * Phy	0.64	3.841	0.8800	1.386	0.79	3.755	0.6800	1.332	0.440	0.635
Ca * Phy	0.49	3.841	0.0200	1.386	0.44	3.755	0.6200	1.332	0.380	0.635
NPP * Ca * Phy	0.83	7.958	0.3900	2.872	0.98	7.780	0.8100	2.759	0.970	1.000
HyD	0.33	1.903	0.1600	0.687	0.63	1.860	0.0300	0.660	0.130	0.314
NPP * Hyd	0.37	3.794	0.0400	1.386	0.39	3.755	0.6300	1.332	0.160	0.635
Ca * HyD	0.40	3.794	0.0400	1.386	0.39	3.755	0.1300	1.332	0.001	0.635
NPP * Ca * HyD	0.59	7.588	0.2000	2.738	0.47	7.780	0.6400	2.630	0.330	1.254
Phy* HyD	0.46	2.683	0.5300	0.974	0.71	2.639	0.9100	0.930	0.280	0.446
NPP * Phy * HyD	0.67	5.498	0.2200	1.984	0.75	5.375	0.2900	1.906	0.740	0.908
Ca * Phy * HyD	0.58	5.498	0.6600	1.984	0.60	5.375	0.9100	1.906	0.830	0.908
NPP * Ca * Phy * HyD	0.48	10.730	0.0400	3.872	0.28	11.492	0.8600	4.075	0.750	1.942
CV	63.03		155.9700		72.15		174.1400		353.750	

Table 7: Effects of main effects on incidence and severity of tibial dyschondroplasia (TD) and on incidence of rickets

Dietary factor		Total Rickets	Calcium Rickets	Phosphorus Rickets	TD Incidence[1]	TD Severity[2]
% Nonphytate P						
	0.35	41.23	5.26	35.98	5.64	1.49
	0.40	45.80	6.67	39.16	5.99	1.80
	0.45	40.26	6.05	34.19	5.02	0.98
	0.50	39.55	6.51	33.01	4.31	0.61
Calcium level						
	- 0.20	44.04	10.46[a]	33.57	9.07[a]	2.50[a]
	2:1	40.64	4.68[b]	35.91	5.20[b]	1.24[b]
	+ 0.20	41.44	4.69[b]	36.78	3.50[b]	0.71[b]
	+ 0.40	40.72	4.67[b]	36.06	3.18[b]	0.44[b]
Phytase supplementation						
	No	43.65	6.05	37.61	5.59	1.31
	Yes	39.77	6.20	33.56	4.88	1.14
Hy-D supplementation						
	No	43.23	6.80	36.42	6.27[a]	1.56
	Yes	40.19	5.44	34.74	4.21[b]	0.89

[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). [ab]Means with common superscripts do not differ significantly (P<0.05).

desired proportion favoring the toe ash performance; otherwise, if only the enzyme or the vitamin D metabolite is supplemented no positive response for toe ash will be obtained. This finding was opposite to that observed by Angel *et al.* (2006) but supported by Biehl *et al.* (1995) when combining $1{,}25(OH)_2D_3$ and phytase. There were no other significant two-way or three-way interactions between nonphytate phosphorus, calcium, phytase and Hy-D on toe ash.

Dietary effects on incidence of rickets and incidence and severity of tibial dyschondroplasia in 18 d broiler chickens: The ANOVA Table showing the probability of the various dietary factors on incidence of total rickets, calcium rickets, phosphorus rickets and incidence and severity of tibial dyschondroplasia (TD) is shown in Table 6. The TD incidence was defined as the percentage of birds that had a TD score greater than 0 (no apparent TD) while the severity of TD was defined as the percentage of birds that had a score of 3 (most severe) as defined by Edwards and Veltmann (1983). The occurrence of rickets was classified as either hypophosphatemic rickets (Long *et al.,* 1984a) or hypocalcemic rickets (Long *et al.,* 1984b). The various factors that have a significant effect on performance are shown on subsequent Tables. Non-significant data is not presented but can be obtained by contacting the authors.

The main effects of the various dietary factors are shown in Table 7. There were no significant effects of nonphytate phosphorus, calcium levels, phytase and Hy-D supplementation on total rickets. Incidence of calcium rickets was significantly influenced by the dietary calcium level. When birds were fed diets with 0.2% less Ca than the 2:1 Ca: NPP ratio, the incidence of calcium rickets was significantly higher than when higher calcium levels were fed with no significant differences among birds fed the other calcium levels. Williams *et al.* (2000) reported a higher incidence of calcium rickets when diets contained high NPP or low Ca which emphasizes not only the association between low dietary calcium levels and rickets but also between unbalanced Ca: NPP ratios and incidence of rickets. In the current study, the diets with 0.2% less Ca than the 2:1 Ca: NPP ratio supplied low calcium and represented a suboptimal Ca:NPP ratio compared to the NRC (1994) recommendation. The incidence of calcium rickets was not significantly influenced by nonphytate phosphorus, phytase supplementation or Hy-D addition. The incidence of phosphorus rickets was not significantly influenced by level of nonphytate phosphorus, calcium level, phytase supplementation or Hy-D addition.

The incidence of TD was not significantly affected by nonphytate phosphorus and phytase supplementation The incidence of TD was significantly affected by both dietary calcium level and supplementation with Hy-D. Birds fed with the lowest calcium level (0.2% less Ca than the 2:1 Ca: NPP ratio) had a significantly higher incidence of TD compared to those fed the other calcium levels. Although no statistical difference was observed between birds fed the higher calcium levels, the incidence of TD tended to be lower as the Ca level increased. Ledwaba and Roberson (2003) observed a higher incidence of TD in birds fed a suboptimal calcium concentration (0.65%) compared to those fed a marginal Ca level (0.85%). During the present study the 0.65% Ca level was only supplied in diets with 0.2% less Ca than the 2:1 Ca: NPP ratio, confirming the results of Ledwaba and Roberson (2003) and suggests that an adequate dietary Ca level is effective in minimizing incidence of TD. A possible mechanism by which low calcium levels increase TD incidence is suggested by Long

Table 8: Effects of two-way interactions among nonphytate phosphorus, calcium, phytase, and Hy-D on percentage of calcium rickets

	----- % Nonphytate P -----			
	0.35	0.40	0.45	0.50
Hy-D x Nonphytate P				
No Hy-D	5.63[abc]	8.67[a]	4.56[c]	8.35[ab]
Plus Hy-D	4.90[abc]	4.67[bc]	7.54[abc]	4.67[bc]
	------- Calcium level -------			
	-0.20%	2:1	+0.20%	+0.40%
Phytase x Calcium level				
No Phytase	8.44[b]	4.06[cd]	4.83[bcd]	6.85[bc]
Plus Phytase	12.48[a]	5.29[bcd]	4.54[cd]	2.48[d]
Hy-D x Calcium level				
No Hy-D	12.63[a]	5.95[bc]	5.54[bc]	3.08[c]
Plus Hy-D	8.29[b]	3.40[c]	3.83[c]	6.25[bc]

[abcd]Means with common superscripts do not differ significantly (P < 0.05).

et al. (1984b) who reported that low calcium levels stimulate the production of 1,25 $(OH)_2D_3$ increasing the intestinal calcium absorption and the calcium renal conservation. This leads to an increased number of viable condrocytes entering the hypertrophied stage and thus, a larger hypertrophied zone. In an ineffective attempt to repair the endochondrial ossification, the tibia with its rapid production and turnover of proliferating condrocytes predispose the TD lesion.

The incidence of TD in birds fed diets containing Hy-D was significantly reduced, contrary to the report by Roberson *et al.* (2005) but in agreement to the reports of Fritts and Waldroup (2003), Ledwaba and Roberson (2003), Zhang *et al.* (1997) and Waldenstedt (2006). The incidence of TD was not significantly affected by level of nonphytate phosphorus or by phytase supplementation. The severity of TD was significantly affected only by the dietary calcium level. Birds fed with 0.2 % less Ca than the 2:1 Ca: NPP had a significantly higher severity of TD than birds fed the higher calcium levels. Although there was no significant difference in TD severity among birds fed the other calcium levels there was a trend to reduced TD severity as the calcium level increased. Severity of TD was not significantly influenced by nonphytate phosphorus, phytase and Hy-D supplementation.

The incidence of calcium rickets was significantly affected by interactions between Nonphytate P and Hy-D, between calcium level and phytase and between calcium level and Hy-D (Table 8). The response of addition of Hy-D to diets with different levels of nonphytate phosphorus was variable. When Hy-D was added to diets with 0.35, 0.40 and 0.50% nonphytate phosphorus the percentage of calcium rickets was reduced, often in a significant manner, but when Hy-D was added to diets with 0.45% Nonphytate P the percentage of calcium rickets was increased.

When phytase was supplemented to diets containing 0.2 % less Ca than the 2:1 Ca: NPP ratio the incidence of calcium rickets was increased. There was little or no effect of adding phytase to diets with the 2:1 Ca: NPP ratio or diets with 0.20% more Ca than the 2:1 ratio. However, addition of phytase to diets with 0.4 % more Ca than the 2:1 Ca: NPP ratio resulted in a significant reduction in incidence of calcium rickets. Phytase supplementation improves the availability of phytate bound phosphorus in the diet and thus may be causing an imbalanced Ca: NPP ratio at the lowest calcium level and improving the Ca: NPP ratio at the highest calcium level. Waldenstedt (2006) mentioned a correct Ca: NPP ratio as a means of preventing rickets and described an abnormal ratio as harmful as a deficiency of either of these minerals.

Table 9: Effect of interaction between nonphytate phosphorus and calcium level on incidence of tibial dyschondroplasia at 18 d[1]

	----- % Nonphytate P -----			
	0.35	0.40	0.45	0.50
- 0.20%	12.88[A]	11.21[AB]	6.86[BC]	5.33[C]
2:1	3.63[C]	6.71[BC]	4.21[C]	6.27[BC]
+ 0.20	3.79[C]	1.88[C]	6.29[BC]	2.04[C]
+ 0.40	2.25[C]	4.17[C]	2.71[C]	3.58[C]

[1]As defined in Table 7. [ABC]Means with common superscripts do not differ significantly (P<0.10).

Table 10: Effect of interaction between calcium level and Hy-D supplementation on severity of tibial dyschondroplasia at 18 d[1]

	----- Calcium level -----			
	-0.20%	2:1	+0.20%	+0.40%
No Hy-D	4.33[a]	1.40[b]	0.15[b]	0.35[b]
Plus Hy-D	0.67[b]	1.08[b]	1.27[b]	0.52[b]

[1]As defined in Table 7. [ab]Means with common superscripts do not differ significantly (P < 0.05).

Addition of Hy-D to diets containing 0.2 % less Ca than the 2:1 Ca: NPP ratio reduced the incidence of calcium rickets, but not when added at higher levels of calcium. This is in agreement with the results of Whitehead (2004) and it is also similar to the observations of Ledwaba and Roberson (2003) who observed that Hy-D was effective in relieving other leg abnormalities such as incidence of TD at calcium concentrations lower than 0.85%. Coincidently, 0.2 % less Ca than the 2:1 Ca: NPP ratio level was the only level supplying less than 0.85% Ca at all the NPP values in the present study. There were no significant interactions observed among nonphytate phosphorus, calcium levels, phytase and Hy-D on incidence of phosphorus rickets.

The incidence of TD was numerically (P=0.09) affected by an interaction between calcium and nonphytate phosphorus (Table 9). Increasing the dietary calcium level tended to reduce the incidence of TD but the reduction was much greater at the lower levels of NPP. At these low levels of NPP the calcium content of diets with 0.20% less Ca than the 2:1 Ca: NPP ratio would be 0.50 to 0.60%, confirming the importance of an adequate Ca level for a proper bone development.

The severity of TD was significantly influenced by an interaction between calcium level and Hy-D supplementation (Table 10). The severity of TD was higher in birds receiving 0.2 % less Ca than the 2:1 Ca: NPP ratio with no Hy-D supplementation than in birds fed higher levels of calcium. Addition of Hy-D significantly reduced the severity of TD when added to diets containing 0.20% less Ca than the 2:1 ratio but not when added to diets with higher calcium levels. This suggests that chicks fed suboptimal calcium levels are more prone to suffer leg abnormalities, which to some extent can be overcome by the addition of the vitamin D metabolite. It also indicates that at adequate calcium levels the addition of Hy-D was not effective at reducing severity of TD. There were no significant three way interactions among the dietary variables for total rickets, calcium rickets, phosphorus rickets, TD incidence and TD severity.

In summary, Ca: NPP ratios and calcium levels similar or even higher than the recommendations of the NRC (1994) are necessary for adequate bird performance. Phytase supplementation improved performance parameters such as FCR and body weight, whereas the Hy-D addition to diets containing an adequate vitamin D level caused a negative effect on FCR due to a possible hypercalcemia condition. Bone development was improved by increasing phosphorus and calcium levels. Moreover, the Hy-D supplementation and its combination with phytase were effective in enhancing bone development. 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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