ISSN 1682-8356
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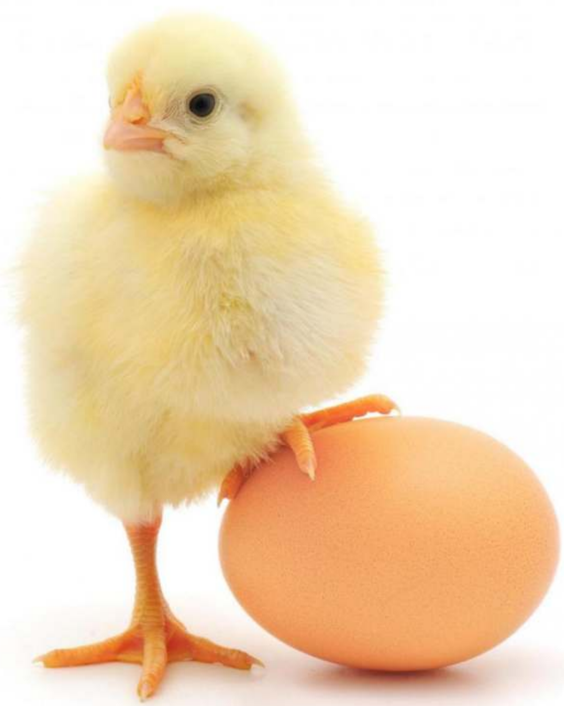

INTERNATIONAL JOURNAL OF
POULTRY SCIENCE

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International Journal of Poultry Science 6 (12): 937-943, 2007
ISSN 1682-8356

Effects of Dietary Levels of Calcium and Nonphytate Phosphorus in Broiler Starter Diets on Total and Water-Soluble Phosphorus Excretion as Influenced by Phytase and Addition of 25-Hydroxycholecalciferol[1]

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Abstract: A study was conducted utilizing a 2 x 2 x 4 x 4 factorial arrangements in which a nutritionally adequate diet was fed with and without 1200 FTU/kg of phytase and with or without 69 µg/kg of 25-hydroxycholecalciferol, four levels of nonphytate phosphorus (NPP) (0.35, 0.40, 0.45, and 0.50%) and four levels of calcium (2:1 Ca:NPP ratio, 0.2% Ca less than the 2:1 ratio, 0.2% Ca more than the 2:1 ratio, and 0.4% Ca more than the 2:1 ratio) for a total of 64 treatments, each fed to two replicate pens of five male broilers in wire-floored battery brooders. At 14 d of age excreta samples were collected, frozen, freeze dried, and analyzed for total P (TP), Ca, and water-soluble P (WSP). The ratio of WSP/TP was calculated from these data. The TP, Ca and WSP in excreta increased as the NPP content of the diet increased. Phytase supplementation reduced TP and Ca but increased WSP concentration and the WSP/TP ratio; this effect might be reversed if levels of NPP lower than those evaluated in the present study are utilized to account for the improvement on phytate phosphorus digestion. The addition of Hy-D reduced TP and Ca concentration in broiler excreta. The most remarkable effect was seen by increasing dietary calcium levels above the 2:1 Ca:NPP ratio typically used in the poultry industry. As the dietary Ca increased, there were significantly reduced excreta levels of TP, WSP and the WSP/TP ratio was significantly reduced. Compared to chicks fed diets with the 2:1 Ca:NPP ratio, the WSP in excreta was reduced 40% by adding 0.20% more Ca and 54% by adding 0.40% more Ca. As the WSP fraction of broiler litter is the primary concern in eutrophication, increasing the dietary Ca level in conjunction with feeding closer to the P requirement should be a cost-effective means of combating the adverse effects of broiler litter on pastures.

Key words: Broilers, phosphorus, eutrophication, calcium, soluble phosphorus, litter

Introduction

The increasing concern about environmental pollution from poultry litter commonly utilized as fertilizer has placed enormous pressure on the poultry industry. Restrictions over certain nutrients have been set in place voluntarily or by law in order to avoid excesses that due to runoff or leaching carry negative effects (Vadas *et al.*, 2004). Phosphorus is considered the major issue among these nutrients because it is recognized as a direct cause of eutrophication impairing water quality (Summers, 1997).

The information related to phosphorus content in litter is generally reported as total phosphorus content (TP). However, many forms of this mineral are found in litter; within them the water soluble phosphorus (WSP) fraction represents the highest potential risk for losses by runoff in agricultural fields (Maguire *et al.*, 2004).

Many nutritional approaches have been developed to reduce phosphorus excretion by poultry such as feeding closer to the requirement, the use of phosphorus sources with high bioavailability, supplementation with phytase enzymes, addition of vitamin D metabolites and the use of modified feedstuffs with low phytate content (Waldroup, 1999). However, their effect on WSP is unclear (Plumstead *et al.*, 2007).

According to Leytem *et al.* (2007) increasing dietary calcium levels reduces the proportion of soluble inorganic phosphorus due to the formation of stable Ca:P complexes that increase the amount of insoluble Ca:P precipitates in litter. The use of exogenous phytase in poultry feeds is becoming more common because of the recent reduction in cost as well as the environmental concern (Summers, 1997). However, the effectiveness of the enzyme on WSP excretion is still a matter of controversy (Maguire *et al.*, 2004). 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 (Fritts and Waldroup, 2003). Some studies report a reduction in phosphorus excreted because of adding vitamin D metabolites (Maguire *et al.*, 2004). It has been suggested that results obtained by many studies evaluating TP and WSP at different NPP levels at a fixed calcium level hide an effect due to the variation of the Ca:P ratio (Leytem *et al.*, 2007).

An evaluation of current industry usage levels of Ca and NPP (Fig. 1) show a broad diversity of inclusion levels in the poultry industry. In order to have a better perspective of the environmental impact of the U.S. poultry industry, it is important to evaluate these nutrient levels and their impact on TP, WSP and the TP/WSP ratio. This study was conducted to evaluate the effect of various levels of Ca and NPP on excretion of calcium and phosphorus in young broilers chickens, as influenced by the addition of phytase and Hy-D.

Materials and Methods

The experimental design for this study consisted first of a 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 contained a commercial vitamin premix that provided 5511 IU/kg of cholecalciferol to the diet. A large batch of this diet was prepared and aliquots supplemented with or without 1200 FTU/kg phytase supplementation (Ronozyme®, DSM Corporation, Parsippany NJ), and with or without 69 µg/kg of Hy-D for a total of four primary test diets. These four primary test diets were analyzed for Ca and total P 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 derived by using NPP levels ranging from 0.35 to 0.50%, in increments of 0.05%. Within each NPP level, four Ca levels were included which are: 1) 2:1 ratio of Ca to NPP, consistent with industry levels shown in Fig. 1; 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. The combination of the 16 levels of Ca and NPP within each of the four primary test diets resulted in 64 dietary treatments. Diets were fed in mash form. Two pens of six male chicks were assigned to each of the 64 test diets.

Male chicks of a commercial strain (Cobb 500[3]) 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 wire 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. At 14 d excreta samples were collected on aluminum foil. Samples were collected from the center of the excreta collection pans to avoid contamination with spilled feed. The excreta samples were frozen, freeze-dried, and ground. Total calcium and phosphorus content were determined by Inductively Coupled Plasma Spectroscopy following HNO_3 digestion. The water-soluble P (WSP) was determined by the method of Self-Davis *et al.* (2000). The ratio of WSP/TP was calculated from these data. At 18 d measurements of body weight, feed conversion, mortality, and bone development were evaluated; these data will be reported elsewhere.

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 and three-way interactions. Percentage data were converted to arc sine prior to analysis. Data are presented as natural number. 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.

Table 1: Composition and calculated analysis of basal diet

Ingredient	g/kg
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	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 $MnSO_4•H_20$) 100 mg; Zn (from $ZnSO_4•7H_2O$) 100 mg; Fe (from $FeSO_4•7H_2O$) 50 mg; Cu (from $CuSO_4•5H_20$) 10 mg; I from $Ca(IO_3)_2•H_20$), 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.

Results and Discussion

The results of the statistical analysis are shown in Table 2. All of the main factors of NPP level, Ca level, addition of phytase, and addition of Hy-D had significance effects on total phosphorus and total calcium levels in broiler excreta, while NPP level, Ca level, and phytase addition had significant effects on total water-soluble phosphorus content. Ca level and phytase supplementation had significant effects on the ratio of water-soluble to total P in excreta. There were few significant interactions among or between the various factors.

Total Phosphorus in excreta: The influence of the various main effects on percentage of phosphorus in excreta is shown in Table 3. There was a significant effect of the NPP level, calcium level, phytase supplementation and Hy-D addition on TP. As expected, TP in the excreta increased as the dietary phosphorus level increased, in agreement with Angel *et al.* (2006), Mitchell and Edwards (1996), Angel *et al.* (2005), Waldroup (1999), Plumstead *et al.* (2007),Viveros *et al.* (2002), Applegate *et al.* (2003), Leytem *et al.* (2007), Summers (1995) and Yan *et al.* (2003). This confirms the importance of feeding close to the P requirement. The TP in excreta was significantly higher at the lowest calcium level with no statistical difference between the remaining levels of dietary calcium. This agrees with Toor *et al.* (2005) but is contrary to Hoek *et al.* (1988) and Al-Masri (1995). A significantly lower TP in excreta was obtained when phytase was added to the diet, in agreement with Toor *et al.* (2005), Mitchell and Edwards (1996), Angel *et al.* (2006), Miles *et al.* (2003), Viveros *et al.* (2002), Maguire *et al.* (2004), Waldroup *et al.* (2000), Nahm (2002), Penn *et al.* (2004), Maguire *et al.* (2005), Leytem *et al.* (2007), Smith *et al.* (2004), Angel *et al.* (2005) and Yan *et al.* (2003) but contrary to the reports by Vadas *et al.* (2004) and Plumstead *et al.* (2007). The latter authors justified this difference because floor pen rearing systems allow more recycling of litter phosphorus than battery cages. Besides, if the dietary NPP level is not effectively reduced when adding phytase, higher TP in litter will be expected. When Hy-D was supplemented the TP in excreta was significantly reduced, confirming the reports of Angel *et al.* (2006), Maguire *et al.* (2004), Mitchell and Edwards (1996) and Biehl and Baker (1997), some of whom utilized a different vitamin D metabolite. Biehl and Baker (1997) suggest that vitamin D metabolites make the phytate complex more vulnerable to the phytase attack enhancing the enzyme function. There were no two-way, three-way, or four-way interactions among NPP level, Ca level, phytase supplementation, or Hy-D supplementation on TP content of broiler excreta.

Calcium in excreta: The influence of various main effects on Ca in excreta is shown in Table 3. Excreta Ca was significantly influenced by the NPP level, calcium level, phytase supplementation, and Hy-D supplementation. When the NPP level was increased, Ca in excreta was significantly increased. This supported by Viveros *et al.* (2002) who mention that at higher levels of NPP more calcium is bound to phytate which cannot be retained and therefore is excreted. As it was expected, more Ca was found in the excreta as the dietary calcium level increased, in agreement with Al-Masri (1995) and Hoek *et al.* (1988) in female rats. The addition of Hy-D to the diet significantly reduced the Ca concentration in the excreta. This effect due to the enhancement of calcium uptake by the vitamin D metabolite is confirmed by Murer and Hildmann (1981), DeLuca (2004) and Jones *et al.* (1998). Phytase supplementation significantly reduced the calcium concentration in excreta. This is supported by Viveros *et al.* (2002) and is possibly due to the release of calcium that binds the phytate molecule.

There was a significant two-way interaction between phytase supplementation and addition of Hy-D on calcium content of excreta (Fig. 2). In the absence of Hy-D, addition of phytase significantly reduced the Ca level in the excreta. When diets were supplemented with Hy-D, there was no further reduction in excreta Ca when phytase was added. This is explained by the positive effect of both Hy-D and phytase at enhancing the uptake and availability of calcium, with no synergistic activity between the two in this respect. There were no other significant interactions among or between the various dietary factors for excreta Ca content.

Water soluble phosphorus in excreta: The influence of various main effects on WSP is shown in Table 3. The WSP in excreta was significantly affected by the NPP level, Ca level, and phytase supplementation. The lowest NPP level rendered a significantly lower amount of WSP, with no significant difference among the remaining levels. Angel *et al.* (2005) and Knowlton *et al.* (2004) also reported higher amounts of soluble phosphorus by feeding higher levels of dietary phosphorus. Moreover, WSP was reduced significantly as the dietary calcium level increased, in agreement with Leytem *et al.* (2007). Tamim *et al.* (2004) observed a marked reduction in the solubility of Ca-phytate complexes at higher pH due to the formation of more stable complexes; as solubility affected the enzymes effectiveness less phytate is hydrolyzed and thus less phosphorus is free. Moreover, Toor *et al.* (2005) affirm that increasing dietary levels of calcium represents a strategy to reduce litter phosphorus through the facilitation of Ca-P interaction forming hydroxyapatite, a complex that is far less soluble than dicalcium phosphate thus reducing the amount of WSP in litter. A significantly higher amount of WSP was observed when

Table 2: ANOVA of total phosphorus (TP), calcium (Ca), water soluble phosphorus (WSP) and WSP/TP ratio in broiler excreta

	TP		Ca		WSP		WSP/TP	
	P>F	SEM	P>F	SEM	P>F	SEM	P>F	SEM
Nonphytate P (NPP)	<0.0001	0.024	<0.0001	0.070	0.0008	134.514	0.35	0.921
Calcium (Ca)	0.01	0.024	<0.0001	0.070	<0.0001	134.514	<0.0001	0.921
Phytase (Phy)	0.02	0.018	0.05	0.050	0.0004	95.590	<0.0001	0.662
Hy-D	<0.0001	0.018	0.01	0.050	0.39	96.590	0.53	0.662
NPP*Ca	0.99	0.049	0.32	0.140	0.64	269.028	0.45	1.843
NPP*Phy	0.77	0.346	0.39	0.099	0.27	201.771	0.31	1.382
NPP*Hy-D	0.93	0.037	0.94	0.105	0.69	201.771	0.74	1.382
Ca*Phy	0.92	0.346	0.34	0.099	0.11	201.771	0.08	1.382
Ca*Hy-D	0.96	0.037	0.81	0.105	0.23	201.771	0.73	1.382
Phy*Hy-D	0.12	0.025	0.03	0.072	0.70	138.654	0.73	0.950
NPP*Ca*Phy	0.75	0.069	0.74	0.198	0.41	380.463	0.58	2.606
NPP*Ca*Hy-D	0.98	0.084	0.50	0.243	0.09	465.970	0.05	3.192
NPP*Phy*Hy-D	0.42	0.055	0.95	0.157	0.87	300.782	0.84	2.060
Ca*Phy*Hy-D	0.36	0.055	0.26	0.157	0.22	300.782	0.42	2.060
NPP*Ca*Phy*Hy-D	0.95	0.978	0.50	0.281	0.77	538.055	0.88	3.686
CV	9.64		19.43		35.55		35.33	

Table 3: Effects of dietary levels of nonphytate P, calcium, phytase supplementation, and addition of Hy-D in broiler starter diets on total P (TP), Ca, water-soluble P (WSP), and ratio of total to water-soluble P in broiler excreta

Dietary factor		TP (%)	Ca (%)	WSP(ppm)	WSP/P, (%)
% Nonphytate P					
	0.35	1.234[d]	1.747[c]	1675.09[b]	13.33
	0.4	1.345[c]	1.776[c]	2123.12[a]	15.53
	0.45	1.525[b]	2.212[b]	2309.31[a]	15.14
	0.5	1.637[a]	2.481[a]	2454.93[a]	14.97
Calcium level					
	-0.2	1.508[a]	1.220[d]	3853.26[a]	25.57[a]
	2:01	1.411[b]	1.644[c]	2279.75[b]	16.50[b]
	0.2	1.418[b]	2.368[b]	1383.89[c]	9.59[c]
	0.4	1.403[b]	2.985[a]	1045.56[c]	7.31[c]
Phytase supplementation					
	No	1.468[a]	2.122[a]	1886.57[b]	12.77[b]
	Yes	1.407[b]	1.987[b]	2394.65[a]	16.71[a]
Hy-D supplementation					
	No	1.497[a]	2.143[a]	2198.93	14.45
	Yes	1.374[b]	1.965[b]	2082.29	15.04

abcdMeans in column with common superscripts do not differ significantly ($P < 0.05$).

phytase was supplemented. This was confirmed by Miles *et al.* (2003) and Toor *et al.* (2005) but is contrary to the reports of Leytem *et al.* (2007) and Smith *et al.* (2004). The fact that in some reports the NPP level was reduced to account for the increasing digestion of phytate phosphorus may be reason to find reduced or no significant impact of phytase on WSP, as reported by Maguire *et al.* (2005), Penn *et al.* (2004), Angel *et al.* (2005) and Maguire *et al.* (2004). Vadas *et al.* (2004) affirm that adding phytase without conservative reduction of phosphorus may not decrease its concentration in litter. This is explained because the phosphorus adsorbed by the phytate molecule is released by phytase increasing the amount of phosphate in the aqueous phase, different to phytate, which is unlikely to be transported in runoff waters. There was no statistical effect of Hy-D addition on the WSP concentration in excreta, confirming the report by Maguire *et al.* (2004). There were no significant interactions among the various factors for WSP content in the excreta.

Ratio of total and water soluble phosphorus: The influence of various main effects on the WSP/TP ratio is shown in Table 3. There were significant effects of the dietary Ca level and phytase supplementation on WSP/TP ratio. Increasing the dietary Ca resulted in a significant reduction in WSP/TP ratio, which is supported by Leytem *et al.* (2007). Birds fed diets with 0.20 and 0.40% Ca greater than a 2:1 Ca:NPP ratio had significantly lower WSP/TP ratios in the excreta than did birds fed the commonly used 2:1 Ca:NPP ratio, with reductions of 42 to 56% in WSP/TP ratio. A significantly higher WSP/TP ratio in excreta was observed when phytase was added, supporting results of Leytem *et al.* (2007) but contrary to the reports by Penn *et al.* (2004), Angel *et al.* (2005) Plumstead *et al.* (2007) and Maguire *et al.* (2005). There was no significant effect of the NPP level or Hy-D supplementation on the WSP/TP ratio.

Table 4: Effects of three-way interaction among nonphytate phosphorus, calcium, and Hy-D in broiler diets on ratio of water-soluble P to total P in broiler excreta

		% Nonphytate P			
% Calcium	Hy-D	0.35	0.4	0.45	0.5
-0.2	No	19.59^{abc}	31.75^{a}	28.22^{a}	24.44^{a}
-0.2	Yes	29.44^{a}	24.49^{abc}	19.85^{abc}	26.75^{ab}
2:01	No	18.45^{cde}	15.98^{def}	16.18^{def}	14.16^{defg}
2:01	Yes	17.59^{cdef}	16.58^{def}	16.97^{def}	16.11^{def}
0.2	No	7.48^{ghi}	8.12^{ghi}	10.24^{fghi}	11.64^{efgh}
0.2	Yes	6.29^{hi}	10.53^{fghi}	11.72^{efgh}	10.71^{fghi}
0.4	No	4.42^{i}	5.05^{hi}	8.11^{ghi}	7.33^{ghi}
0.4	Yes	3.39^{i}	11.78^{efgh}	9.81^{fghi}	8.59^{ghi}

abcdefghiMeans with common superscripts do not differ significantly ($P < 0.05$).

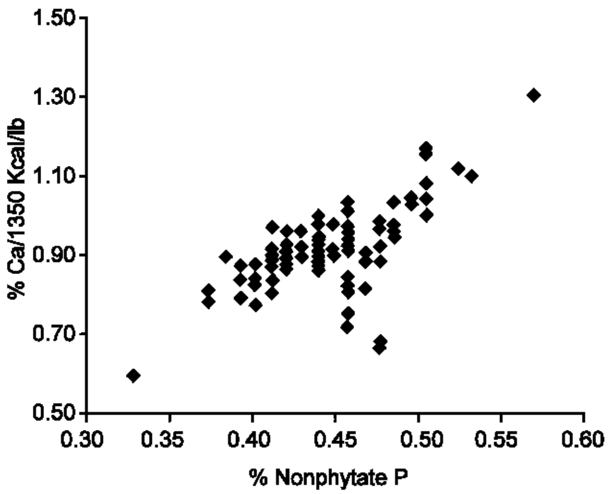

Fig. 1: Current industry usage levels of Ca and AP levels in broiler starter diets (Survey by Agri Stats, Fort Wayne IN)

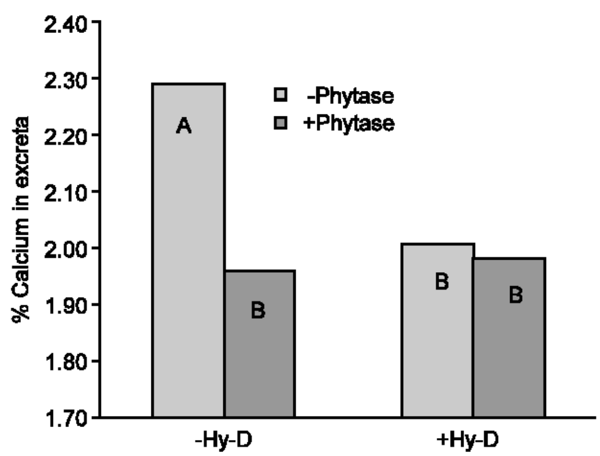

Fig. 2: Interaction between dietary supplementation with phytase and Hy-D on calcium content of broiler excreta

There was a significant interaction among NPP levels, Ca level, and Hy-D supplementation (Table 4). When HyD was added to the diet it increased the WSP/TP ratio at some levels of Ca and NPP and reduced it at other levels; however there did not appear to be any consistency in its response, resulting in the significant interaction. There were no other significant interactions among or between the various dietary factors on WSP/TP ratios.

In summary, TP, Ca and WSP in litter increased as the NPP content of the diet increased. Phytase supplementation reduced TP and Ca but increased WSP concentration and the WSP/TP ratio; this effect might be reversed if levels of NPP lower than those evaluated in the present study are utilized to account for the improvement on phytate phosphorus digestion. The addition of Hy-D reduces TP and Ca concentration in broiler excreta. Perhaps the most remarkable effect was seen by increasing dietary calcium levels above the typical 2:1 Ca:NPP ratio that is typically used in the poultry industry. As the dietary Ca increased there were significantly reduced levels of TP, WSP and the WSP/TP ratio. Compared to chicks fed diets with the 2:1 Ca:NPP ratio, the WSP in excreta was reduced by 40% by adding 0.20% more Ca and by 54% by adding 0.40% more Ca. The increased dietary calcium levels had no negative effect on live performance and bone mineralization (Coto, 2007). As the WSP fraction of broiler litter is the primary concern in eutrophication, increasing the dietary Ca level in conjunction with feeding closer to the P requirement should be a cost-effective means of combating the adverse effects of broiler litter on pastures.

References

Al-Masri, M.R., 1995. Absorption and endogenous excretion of phosphorus in growing broiler chicks, as influenced by calcium and phosphorus ratios in feed. Br. J. Nutr., 74: 407-415.

Angel, C.R., W. Powers, T. Applegate, N. Tamim and M. Christman, 2005. Influence of phytase on water-soluble phosphorus in poultry and swine manure. J Environ. Qual., 34: 563-571.

Angel, R., W.W. Saylor, A.D. Mitchell, W. Powers and T.J. Applegate, 2006. Effect of dietary phosphorus, phytase, and 25-hydroxycholecalciferol on broiler chicken bone mineralization, litter phosphorus, and processing yields. Poult. Sci., 85: 1200-1211.

Applegate, T., B. Joern, D. Nussbaum-Wagler and R. Angel, 2003. Water-soluble phosphorus in fresh broiler litter is dependent upon phosphorus concentration fed but not on fungal phytase supplementation. Poult. Sci., 82: 1024-1029.

Biehl, R.R. and D.H. Baker, 1997. Utilization of phytate and nonphytate phosphorus in chicks as affected by source and amount of vitamin D3. J. Anim. Sci., 75: 2986-2993.

Coto, C.A., 2007. Interrelationship of dietary levels of calcium and phosphorus with phytase and 25-hydroxycholecalciferol in diets for broiler chickens. M.S. Thesis, University of Arkansas, Fayetteville AR.

DeLuca, H.F., 2004. Overview of general physiologic features and functions of vitamin D. Am. J. Clin. Nutr., 80: 1689S-1696S.

FASS, 1999. Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching. 1st rev. ed. Federation of Animal Science Societies, Savoy, IL.

Fritts, C.A. and P.W. Waldroup, 2003. Effect of source and level of Vitamin D on live performance and bone development in growing broilers. J. Appl. Poult. Res., 12: 45-52.

Hoek, A.C., A.G. Lemmens, J.W.M.A. Mullink and A.C. Beynen, 1988. Influence of dietary calcium:phosphorus ratio on mineral excretion and nephrocalcinosis in female rats. J. Nutr., 118: 1210-1216.

Jones, G., S.A. Strugnell and H.F. DeLuca, 1998. Current understanding of the molecular actions of vitamin D. Physiol. Rev., 78: 1193-1231.

Knowlton, K.F., J.S. Radcliffe, C.L. Novak and D.A. Emmerson, 2004. Animal management to reduce phosphorus losses to the environment. J. Anim. Sci., 82: E173-195.

Leytem, A.B., P.W. Plumstead, R.O. Maguire, P. Kwanyuen and J. Brake, 2007. What aspect of dietary modification in broilers controls litter water-soluble phosphorus: Dietary phosphorus, phytase, or calcium? J. Environ. Qual., 36: 453-463.

Maguire, R.O., Z. Dou, J.T. Sims, J. Brake and B.C. Joern, 2005. Dietary strategies for reduced phosphorus excretion and improved water quality. J. Environ. Qual., 34: 2093-2103.

Maguire, R.O., J.T. Sims, W.W. Saylor, B.L. Turner, R. Angel and T.J. Applegate, 2004. Influence of phytase addition to poultry diets on phosphorus forms and solubility in litters and amended soils. J. Environ. Qual., 33: 2306-2316.

Miles, D., P. Moore Jr, D. Smith, D. Rice, H. Stilborn, D. Rowe, B. Lott, S. Branton and J. Simmons, 2003. Total and water-soluble phosphorus in broiler litter over three flocks with alum litter treatment and dietary inclusion of high available phosphorus corn and phytase supplementation. Poult. Sci., 82: 1544-1549.

Mitchell, R.D. and H.M. Edwards Jr., 1996. Additive effects of 1,25-dihydroxycholecalciferol and phytase on phytate phosphorus utilization and related parameters in broiler chickens. Poult. Sci., 75: 111-119.

Murer, H. and B. Hildmann, 1981. Transcellular transport of calcium and inorganic phosphate in the small intestinal epithelium. Am. J. Physiol. Gastrointest. Liver Physiol., 240: G409-416.

Nahm, K.H., 2002. Efficient feed nutrient utilization to reduce pollutants in poultry and swine manure. Crit. Rev. Environ. Sci. Technol., 32: 1-16.

Penn, C.J., G.L. Mullins, L.W. Zelazny, J.G. Warren and J.M. McGrath, 2004. Surface runoff losses of phosphorus from Virginia soils amended with turkey manure using phytase and high available phosphorus corn diets. J. Environ. Qual., 33: 1431-1439.

Plumstead, P.W., H. Romero-Sanchez, R.O. Maguire, A.G. Gernat and J. Brake, 2007. Effects of phosphorus level and phytase in broiler breeder rearing and laying diets on live performance and phosphorus excretion. Poult. Sci., 86: 225-231.

SAS Institute, 1991. SAS User's Guide: Statistics. Version 6.03 ed. ed. SAS Institute, Inc., Cary, NC.

Self-Davis, M.L., P.A. Moore, Jr. and B.C. Joern, 2000. Determination of water- and/or dilute salt-extractable phosphorus. Pages 24-26 in: Methods of phosphorus analysis for soils, sediments, residuals, and waters. Southern Cooperative Series Bulletin No. 396. G. M. Pierzunski, ed. North Carolina State University, Raleigh, NC.

Smith, D.R., P.A. Moore Jr., D.M. Miles, B.E. Haggard and T.C. Daniel, 2004. Decreasing phosphorus runoff losses from land-applied poultry litter with dietary modifications and alum addition. J. Environ. Qual., 33: 2210-2216.

Summers, J.D., 1995. Reduced dietary phosphorus levels for layers. Poult. Sci., 74: 1977-1983.

Summers, J. D. 1997. Precision phosphorus nutrition. J. Appl. Poult. Res., 6: 495-500.

Tamim, N., R. Angel and M. Christman, 2004. Influence of dietary calcium and phytase on phytate phosphorus hydrolysis in broiler chickens. Poult Sci., 83: 1358-1367.

Toor, G., J.D. Peak and J.T. Sims, 2005. Phosphorus speciation in broiler litter and turkey manure produced from modified diets. J. Environ. Qual., 34: 687-697.

Vadas, P.A., J.J. Meisinger, L.J. Sikora, J.P. McMurtry and A.E. Sefton, 2004. Effect of poultry diet on phosphorus in runoff from soils amended with poultry manure and compost. J. Environ. Qual., 33: 1845-1854.

Viveros, A., A. Brenes, I. Arija and C. Centeno, 2002. Effects of microbial phytase supplementation on mineral utilization and serum enzyme activities in broiler chicks fed different levels of phosphorus. Poult. Sci., 81: 1172-1183.

Waldroup, P.W., 1999. Nutritional approaches to reducing phosphorus excretion by poultry. Poult. Sci., 78: 683-691.

Waldroup, P.W., J.H. Kersey, E.A. Saleh, C.A. Fritts, F. Yan, H.L. Stilborn, R.C. Crum Jr. and V. Raboy, 2000. Nonphytate phosphorus requirement and phosphorus excretion of broiler chicks fed diets composed of normal or high available phosphate corn with and without microbial phytase. Poult. Sci., 79: 1451-1459.

Yan, F., C.A. Fritts and P.W. Waldroup, 2003. Evaluation of modified dietary phosphorus levels with and without phytase supplementation on live performance and fecal phosphorus levels in broiler diets. 1. Full-term feeding recommendations. J. Appl. Poult. Res., 12: 174-182.

[1]Published with approval of the Director, Arkansas Agricultural Experiment Station, Fayetteville AR 72701. Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the University of Arkansas and does not imply its approval to the exclusion of other products that may be suitable. This project was supported by a grant from Cobb-Vantress Inc., Siloam Springs AR.

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