Various Levels of Calcium and Phosphorus Diets in Response to 1, 25 – Dihydroxycholecalciferol in Laying Hens

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ABSTRACT

The effect of 1,25-dihydroxycholecalciferol [1,25-$(OH)_2 D_3$] supplementation on laying hen diets was evaluated using diets high and low in Ca (3.0% and 1.8%) and high and low in P (0.52% and 0.33%). Since two levels of 1,25- $(OH)_2 D_3$ supplementation (0 and 5 µg/kg diet) were applied, the experiment was a 2 X 2 X 2 factorial arrangement in a completely randomized design using nine individually housed hens per each 8 treatments (72 in total). The hens were fed the diets for 28 days and records were kept on body weight, egg production, feed intake, egg weight, and egg specific gravity. At the end of the experiment, the hens were bled for plasma Ca and P determination and after being euthanased the left tibia removed for bone ash measurement. The corn-soybean meal based diet contained 0.1% Cr_2O_3 as an indicator for determining Ca, P and phytate phosphorus retention at 14 and 28 days. 1, 25-Dihydroxycholecalciferol supplementation had no effect on hen weight, egg production or feed intake. However, large increases in egg specific gravity were obtained when 1, 25-$(OH)_2 D_3$ was fed to hens receiving the low Ca diet. All of the treatments had significant effects on bone ash. The greatest effect of 1, 25-$(OH)_2 D_3$ on bone ash was obtained in those hens fed the high Ca and P diet that was then supplemented with 1,25-$(OH)_2 D_3$ (49.3% vs 53.9% bone ash). At day 14, the high Ca diets decreased phytate P retention while at d 28 the high P diets decreased phytate P retention.

Keywords: Calcium, 1, 25-dihydroxycholecalciferol, Phytate phosphorus, Phosphorus.

INTRODUCTION

When laying hens were fed a corn-soybean meal based diet, they utilized only 8% of the phytate P present in that diet as measured by phytate disappearance (Nelson, 1976). This finding is in agreement with the poor utilization of phytate P by laying hens reported by Gillis *et al*. (1953). Sebastian *et al*. (1998) listed the following as factors affecting phytate phosphorus utilization: dietary calcium and phosphorus concentration, dietary vitamin D_3 concentration, age of bird, phytase activity of dietary ingredients, fiber and genotype. Qian *et al* (1997) determined that calcium retention ranged between 42% and 67%, depending on the calcium: total phosphorus ratio, the addition of 66 or 666 µg kg^{-1} diet vitamin D_3, and the addition of 0-900 phytase units kg^{-1} diet. No critical studies have been conducted on the effect of dietary Ca and P levels on the utilization of phytate P by laying hens. Recently it was found that the addition of the vitamin D_3 derivative 1, 25-dihydroxycholecalciferol (1, 25-$(OH)_2 D_3$) to a corn-soybean meal diet for broilers stimulated phytate P utilization (Edward, 1993).

The experiment reported in this paper was conducted to determine whether supplementation of corn-soybean meal laying diets with 5 mg of a 1, 25-$(OH)_2 D_3$ / kg diet would stimulate natural phytate utilization at adequate and low levels of dietary Ca and P.

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MATERIAL AND METHODS

Seventy-two Single Comb White Leghorn hens were selected from among 108 sixty week-old hens during a 10 day pre-experiment period. The hens were allocated on the basis of their egg production in 24 pens with three individual cages equalizing average egg production within the pens. The corn-soybean meal based diet presented in Table 1 was utilized during a 28 day period. All substitutions were made at the expense of corn.

Limestone (39%Ca) was utilized as a source of calcium. Commercial dicalcium phosphate (analyzed 23.5%Ca and 19%P) was used as a P and Ca source. Two levels of Ca (3.0% and 1.8%) with two levels of total P (0.52% and 0.33%) and two levels of $1,25\text{-}(OH)_2$ D_3 (0 and 5µg/kg) were used in a 2 X 2 X 2 factorial arrangement in a completely randomized design. The hens were allowed to consume feed and water *ad libitum.* Chromic oxide added to the all treatment diets as an indicator. Collection of excreta was carried out twice every two weeks for 48 hours during the 28 days of the experiment. This was accomplished by setting

Table 1. Composition of basal ration

Ingredient	%
Ground yellow corn	75.23
Soybean meal	22.00
Poultry oil	2.00
Salt (sodium chloride)	0.30
Vitamin premix[a]	0.25
Trace mineral premix[b]	0.05
DL-methionine	0.07
Chromic oxide	0.10

[a] Vitamin premix provides (per kg of diet): vitamin A, 5, 500 IU; vitamin D_3, 1,100 ICU; vitamin E, 11 IU; riboflavin,4.4 mg; calcium pantothenate,12 mg; nicotinic acid, 44 mg; choline cl, 220 mg; vitamin $B_{12,}$ 9 µg; vitamin B_6,3.0 mg; menadione, 1.1mg (as menadione sodium bisulfite); folic acid, 3 mg; d-biotin. 3 mg; thiamine, 2.2 mg (as thiamine mononitrate); ethoxyquin, 125 mg.
[b] Trace mineral premix provides (PPm of diet): MnO_2 111; Z no, 75; $FeSO_4$ $7H_2O$ 100; $CuSO_4$,14; $(Io_3)2$, 7.5; Ca 7.5; $FeCO_{3,}$ 41.5.

individual trays under each hen. Dried feed and excreta samples were analyzed for Ca, P and phytate phosphorus by modified HPLC method of Bos *et al* (1991) and the percent retention of Ca, P and phytate phosphorus was calculated. At the end of the experiment, the hens were individually weighed and bled by heart puncture for analyzing plasma Ca and P levels. They were then euthanased by cervical dislocation and their left tibia was removed for bone ash determination based on fat-free by method (Association of Official Agricultural Chemists, 1955).

Egg production was recorded daily and feed consumption was measured at the end of the experiment. Egg specific gravity based on the flooding method and egg weight were determined weekly. From these data, average daily feed consumption per hen and, feed required per unit of egg weight were calculated. All statistical analyses were conducted on pen average. Analyses of data were computed using the SAS analysis of variance procedure (Helwing and Council, 1979). Main effects were separated by Duncan's multiple range test.

RESULTS

There were no significant treatment effects on hen body weight (BW) or feed intake. These data are not presented. Only the Ca level caused a significant effect on feed/g egg (g/g). The birds receiving the high Ca diet required 2.0 g feed/g egg and those receiving the low Ca diets consumed 2.4 g feed/g egg, these data are not presented. Data on weekly egg production, egg weight and egg specific gravity were analyzed using the week as a continuous variable. The egg production data showed significant effects for both diet and week with no interaction, indicating that the significant effect of the low Ca diet in reducing egg production was just an acceleration of the general decline in egg production by all treatments during the experiment (Table 2). Neither the level of P nor the addition to the diet of the 5 mg/kg of

Table 2. Effect of dietary 1,25-dihydroxycholecalciferol (1,25-(OH)$_2$D$_3$), Ca and P on egg production, Egg weight and specific gravity, plasma Ca and P, bone ash and phytate P retention by laying hens

Ca Dietary Content	P Content	1,25-(OH)$_2$D$_3$	Egg production 4 wk	Egg wt 4wk	Egg specific gravity 4wk	Plasma minerals Ca	Plasma minerals P	Boneash	Phytate P retention 14 d	Phytate P retention 28 d
(%)	(%)	(μg/kg)	(%)	(g)		((μg/dL))	((μg/dL))	(%)	(%)	(%)
1.8	0.33	0	67[abc]	64.0	1.069	26.7[ab]	1.02[c]	50.1[cd]	39.9[a]	43.0[a]
1.8	0.52	0	60[c]	65.9	1.076	24.6[ab]	3.05[ab]	51.2[cd]	31.3[ab]	38.3[ab]
3.0	0.33	0	72[ab]	64.7	1.070	22.3[b]	0.91[c]	49.3[d]	27.3[ab]	34.0[ab]
3.0	0.52	0	76[a]	63.2	1.069	31.4[a]	2.44[abc]	55.2[a]	11.5[b]	26.5[ab]
1.8	0.33	5	58[c]	66.1	1.077	29.5[ab]	1.76[cb]	49.8[cd]	34.3[ab]	40.4[a]
1.8	0.52	5	64[bc]	65.1	1.079	25.4[ab]	3.20[ab]	51.8[cb]	34.9[ab]	29.6[ab]
3.0	0.33	5	72[ab]	64.4	1.069	27.4[ab]	1.10[c]	53.9[ab]	25.4[ab]	41.3[a]
3.0	0.52	5	74[ab]	64.4	.078	27.8[ab]	3.80[a]	56.3[a]	17.8[ab]	10.7[b]
Mean ± SEM			68 ±7	64.7±1.2	1.073±0.002	26.9±2.1	2.2±0.5	52.2±0.8	27.8±8.0	33.0±8.8

ANOVA(probabilities)

Effect	df	Egg production	Egg wt	Egg specific gravity	Plasma Ca	Plasma P	Boneash	14 d	28 d
Treatment	7	0.007	0.071	<0.001	0.165	0.002	<0.001	0.155	0.242
Calcium (Ca)	1	<0.001	0.004	<0.001	0.665	0.561	0.001	0.010	0.138
Phosphorus (P)	1	0.484	0.402	0.699	0.581	0.001	0.001	0.135	0.047
Ca X P	1	0.495	0.187	0.938	0.019	0.577	0.027	0.453	0.378
1,25-(OH)$_2$D$_3$	1	0.495	0.041	0.354	0.422	0.087	0.012	0.907	0.435
Ca X 1,25-(OH)$_2$D$_3$	1	0.895	0.458	0.074	0.744	0.621	0.020	0.749	0.911
P X 1,25-(OH)$_2$D$_3$	1	0.252	0.797	0.098	0.098	0.673	0.225	0.393	0.256
Ca X P X 1,25-(OH)$_2$D$_3$	1	0.112	0.469	0.098	0.290	0.206	0.048	0.960	0.505
Week		0.023	0.137	0.290	--	--	--	--	--

Means with different superscripts are significantly different (p< 0.1).

1,25-(OH)$_2$ D$_3$ had any effect on egg production. The week had no effect on egg weight or egg specific gravity. Higher Ca levels in the diet and zero 1,25-(OH)$_2$ D$_3$ supplementation both resulted in significantly improved egg weight. The higher Ca level also caused a significant increase in egg specific gravity. A significant interaction between Ca level and 1,25-(OH)$_2$ D$_3$ supplementation was obtained. Large increases in specific gravity were obtained when 1,25-(OH)$_2$ D$_3$ was fed to the hens receiving the low Ca diet. While the individual levels of Ca and P had no significant effect on the plasma Ca level, there was a significant interaction; raising the P level of the low Ca diet caused a decrease in plasma Ca, while raising the P content of the high Ca diet caused an increase in plasma Ca. Only the P level in the diet caused a significant effect on blood P; low plasma P levels were noted when the low P diet was fed.

All of the individual treatments had a significant effect on bone ash and only one of the interactions (P X 1,25-(OH)$_2$ D$_3$) was not significant. The higher bone ash values were obtained from hens fed the high levels of both Ca and P. The greatest effect of 1,25-(OH)$_2$ D$_3$ on bone ash was apparent in those hens fed the high Ca and low P diet that was then supplemented with 1,25-(OH)$_2$ D$_3$ (49.3% vs 53.9% bone ash).

There is considerable in the phytate P retention data obtained for 14 days and 28 days. In the 14 days data, the most striking effect was the significant decrease in phytate P retention when the high Ca level was present in the diet. While, in the 28 days data, the P level of the diets was the significant factor; at every dietary Ca and 1,25-(OH)$_2$ D$_3$ level, increasing dietary P caused a decrease in phytate P utilization.

DISCUSSION

There were sufficient significant effects of 1,25-(OH)$_2$ D$_3$ supplementation on the diverse criteria measured to indicate definite physiological effects. However, the lack of effect of 1,25-(OH)$_2$ D$_3$ supplementation on many criteria, while significant interacts were obtained for the same criteria, indicates the importance of dietary Ca and P levels in any studies of the effects of this compound in laying hens. Harms *et al.,* 1990, conducted several different experiments that explored individually the effect of high and low Ca or P on the response of hens to 1,25-(OH)$_2$ D$_3$ .The bone ash data obtained by Harms *et al.,* 1990 is similar in most respects to the data obtained in the complete factorial in the paper. When hens were fed a high Ca (3.25%) and low P (0.38%) diet their tibial ash increased from 59.5% to 60.4% and bone breaking strength increased from 4.54 to 6.36 when 1,25-(OH)$_2$ D$_3$ was fed. This would be comparable to the results in this trial when 3.0% Ca and 0.33% P were fed and 1,25-(OH)$_2$ D$_3$ supplementation resulted in a bone ash increase from 49.3% to 53.9%. Harms *et al.,* (1990), also noted that 1,25-(OH)$_2$ D$_3$ supplementation of the high Ca (3.25%) low P diet (0.38%) resulted in a significant decrease in plasma Ca while a slight increase in plasma Ca resulted from 1,25-(OH)$_2$ D$_3$ supplementation in the present study. This, of course, could have been caused by small differences in sampling times as related to oviposition (Sooncharernying and Edwards, 1989; Frost *et al.,* 1990) between the different studies. Supplementation of laying diets that are adequate in Ca and P with 1,25-(OH)$_2$ D$_3$ both in the present study and in other studies (Harms *et al.,*1988; Frost *et al.,*1990; Harms *et al.,*1990) dose not significantly increase tibial ash.

It is interesting that the hens receiving the high Ca diets, had significantly lower 14 days phytate P retention, while those receiving the high P diets had significantly lower 28 days phytate P retention. Concentration of both of these nutrients is known to influence phytate P utilization in broilers (Nelson, 1969; Mitchell and Edwards, 1996). In a similar manner, high dietary Ca and P results in a decrease and increase, respectively, in the retention of phytate P.

Even though the retention of phytate P was not significantly improved by adding 1,25-$(OH)_2$ D_3 to the diets of hens receiving the high level of Ca (3.0%) and low P (0.33%), the increase in bone ash was very great. In this case, future studies on the effect of 1,25-$(OH)_2$ D_3 on phytate utilization should probably be conducted with the same diet.

REFERENCES

1. Association of official Agricultural Chemists, 1955. *Official Methods of Analysis*, 8th ed. Association of Official Agricultural Chemists, Washington, D. C.

2. Bos, K. D., Verbeck, C., van Emden C.H.P., Slump, P. and Wolters, M.G.E. 1991. Improved Determination of Phytate by Ion-exchange Chromatography. *J. Agr. Food Chemisry*. **39**: 1770-1772.

3. Edwards, H. M., and Hardy, M. 1993. Dietary 1, 25- hydroxycholecalciferol Supplementation Increases Natural Phytate Phosphorus Utilization in Chickens. *J. Nutr.* **123**: 567-577.

4. Frost, T. J., and Roland, D.A. 1990. Influence of vitamin D_3, 1 - hydroxyvitamin D_3, and 1,25-dihydroxyvitamin D_3 Eggshell Quality, Tibia Strength, and Various Production Parameters in Commercial Laying Hens. *Poultry Sci.* **69**: 2008-2016.

5. Gillis, M. B., Norris, L. C. and Heuser, G. F. 1953. Phosphorus Metabolism and Requirement of Hens. *Poultry Sci.* **32**: 977-984.

6. Harms, R. H., Wilson, H. R. and Miles, R. D. 1988. Research Note: Influence of 1,25-dihydroxyvitamin D_3 on the Performance of Commercial Laying hens. *Poultry Sci.* **67**: 1233-1235.

7. Harms, R. H., Bootwalla, S. M. Woodward, S.A. and Wilson, H. R. 1990. Some Observations on the Influence of Vitamin D Metabolites when Added to the Diet of Commercial Laying Hens. *Poultry sci.* **69**: 426-432.

8. Helwing, J. T., and Council, K. A.1979. *SAS® User's Guide*. SAS Institute Inc., Raleigh, N.C.

9. Mitchell, R. D., and Edwards, H. M. 1996. Effects of Phytase and 1,25-dihydroxycholecalciferol on Phytate Utilization and the Quantitative Requirement for Calcium and Phosphorus in Young Broiler Chickens. *Poultry Science*. **75**: 95-110

10. Nelson, T. S. 1976. The Utilization of Phytate Phosphorus by poultry- a review. *Poultry Sci*. 46:862-871.

11. Nelson, T.S., 1969. The hydrolysis of phytate phosphorus by chicks and laying hens. *Pouty Sci.* **55**: 2262-2264.

12. Qian, H., Kornergay, E.T. and Denbow, D. M. 1997. Utilization of Phytate Phosphorus and Calcium as Influenced by Microbial Phytase, Cholecalciferol, and the Calcim: Total Phosphorus Ratio in Broiler Diets. *Poultry Sci.* **76**: 37-46.

13. Sebastian, S., Touchburn, S. P. and Chavez, E. R. 1998. Implications of Phytic Acid and Supplemental Microbial Phatase in Poultry Nutrition: a Review. *World Poultry Sci. J.* **54**: 27-47.

14. Sooncharernying, S., and Edwards, H. M. 1989. Effect of Dietary Calcium and Phosphorus Levels on Ultra-filterable Calcium and Dialyzable Phosphorus in the Laying hen. *Poultry Sci.* **68**: 79-723.

اثر افزودن ۱-۲۵- دی هیدروکسی کوله کلسیفرول در جیره حاوی سطوح مختلف کلسیم و فسفر مرغهای تخم‌گذار

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چکیده

اثر افزودن دو سطح (صفر و ۵ میکروگرم در کیلوگرم) ۱-۲۵- دی هیدروکسی کوله کلسیفرول به جیره مرغان تخم گذر حاوی سطوح بالا و پایین کلسیم (۳٪ و ۱/۸ ٪) و فسفر (۰/۵۲ ٪ و ۰/۳۳ ٪) در یک طرح کاملا تصادفی به روش فاکتوریل ۲×۲×۲ با ۸ تیمار و ۹ مرغ تخم‌گذار برای هر تیمار در قفسهای انفـرادی (جمعا" ۷۲ مرغ) مورد مطالعه قرار گرفت. مرغها جیره‌های آزمایشی را به مـدت ۲۸ روز تغذیـه نمودنـد و اطلاعات مربوط به تغییرات وزن بدن, تولید تخم مرغ, مصرف غذا, وزن تخم مرغ و وزن مخصوص تخم مرغ مورد تجزیه و تحلیل آماری قرار گرفت. در پایان آزمایش خونگیری از مرغها انجام و سطح کلسیم و فسفر پلاسمای خون تعیین و پس از کشتار, استخوان ساق پای چپ آنها جهت تعیین خاکستر استخوان ساق جدا گردید. جیره‌های آزمایشی حاوی ذرت وسویا بود و مقدار ۰/۱ ٪ اکسید کرم بـه عنـوان نشـانگر جهت تعیین میزان ابقا کلسیم, فسفر و فسفر فیتیک در روزهای ۱۴ و ۲۸ آزمایش به آن افزوده شد. افزودن ۱-۲۵- دی هیدروکسی کوله کلسیفرول هیچگونه اثر معنی داری روی وزن مرغهـا, تولیـد تخم مـرغ و مصرف غذای آنها نداشت اما باعث افزایش وزن مخصوص تخم مرغ در مرغهـای تغذیـه شـده بـا کلسـیم پایین گردید. اثر تمام تیمارها روی خاکستر استخوان معنی دار بود و بیشترین تاثیر مربوط به افزودن ۱-۲۵- دی هیدروکسی کوله کلسیفرول به جیـره حـاوی کلسـیم و فسـفر بـالا بـود (۴۹/۳٪ در مقابـل ۵۳/۹٪). در روزهای ۱۴ و ۲۸ بترتیب جیره حاوی کلسیم بالا و فسفر بالا ابقا فسفر فیتیک در بدن را کاهش دادند.