ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF POULTRY SCIENCE

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International Journal of Poultry Science 4 (7): 497-499, 2005
ISSN 1682-8356

The Influence of Dietary Phosphorus Level on Plasma Calcium and Phosphorus, Eggshell Calcium and Phosphorus

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Abstract: This study was conducted to determine the effect of dietary nonphytate phosphorus level and vitamin-D_3 on serum and eggshell calcium and phosphorus concentrations of laying hens in late production period. One hundred ninety two 70 weeks white Lohman LSL laying hens were randomly assigned to eighty groups equally (n = 24) each treatment was replicates six times. Experimental diets were prepared by adding non phytate phosphorus at the levels of 0, 0.15, 0.30 and 0.45% to basal diet with and without vitamin D_3 (3000 IU/kg) respectively. Study was lasted for 8 weeks. All diets were isocaloric and isonitrogenous. Serum calcium and phosphorus, eggshell calcium and phosphorus concentrations were evaluated in this study. All parameters were measured the serum Ca and P, eggshell Ca and P levels were influenced from dietary treatments by statistically significant ($P < 0.01$). NPP supplementation decreased serum Ca levels, incontrast, increased eggshell Ca levels linearly as increasing dietary NPP levels. NPP supplementation also increased both serum and eggshell P levels, when vitamin D_3 was omitted from diet. On the other hand, vitamin D_3 supplementation caused some significant changes on Ca and P levels in serum and eggshell content. Vitamin D_3 supplementation increased both serum Ca and eggshell Ca, but, decreased serum P level. On the other hand, vitamin D_3 supplementation significantly increased eggshell P in first two groups but decreased the eggshell P in following groups. Serum phosphorus was directly related to dietary phosphorus levels whereas, serum calcium was inversely related to dietary phosphorus levels.

Key words: Serum Ca, serum P, eggshell Ca and P, laying hen

Introduction

Previous studies have established that phosphorus is an essential nutrient for laying hens because of its role in egg shell formation and metabolism (Said *et al.*, 1984; Roa *et al.*, 1992). Phosphorus may affect more biological systems than any other element. It is an important element in many body functions including bone formation, acid-base balance, metabolism of fat and carbonhydrates, egg formation and in the proteins, carbonhydrates and lipids used throughout the body (Higwill and Winged, 1998).

But resent data indicates that phosphorus in the laying hens diet greater than that needed for maximum egg production is detrimental to egg shell quality (Milles *et al.*, 1983). However, there are considerable variations in NPP recommendations for the laying hens. Sohail and Ronald (2002) mentioned that NPP requirement for commercial leghorns has constantly declined during the last 40 yr., and the NRC recommendation on NPP has been reduced from 429 mgr per hen/d in 1960 to 350 mgr per hen/d in 1984. In 1994 NRC further reduced the dietary NPP requirements for white leghorns from 350 mgr per hen/d (NRC, 1984) to 250 mgr per hen/d (1994 NRC). But can utilize only one-third of the P contained feedstuffs of plant origin (Cromwell, 1989) an improvement in the utilization of pyhtin P will reduce the cost of adding inorganic P sources in the feeds lower the P excretion in the manure and subsequently reduce pollution problems (Nahasol *et al.*, 1994) with regard to. The main mechanism by which vitamin D facilitates calcification of bone and formation of egg shell is believed to be a result of the effects of the physiologically active form of vitamin D, 1,25-Dhydroxycholecalciferol [1,25 $(OH)_2$ D_3] on intestinal function (Vaiano *et al.*, 1994). It is well established that in laying hens a vitamin D_3 dependent Ca-binding protein is involved in the active transport of Ca across the intestinal membrane and probably across the uterine membrane. Dietary and endogenous vitamin D_3 is first hydroxylated position 25 of the vitamin D_3 molecule in the liver to produce 25-OH cholecalciferol (25-OH-D_3) which is the main circulating vitamin D_3 metabolite in the blood. The circulating 25-OH-D_3 is then further hydroxylated in position of the molecule in the kidney to produce 1,25 $(OH)_2$ D_3. this active form of vitamin D_3 is involved in the biosyntheses of Ca-binding protein, which is involved in active transport of Ca across the intestinal wall. This active form is promoting absorption of Ca for bone and egg shell formation (Stevens and Blair, 1984, Vaiona *et al.*, 1994, Keshavarz, 2003). 1,25 $(OH)_2D_3$ also influence alkaline phosphates activity in duodenal mucosa (Grunder and Tsany, 1990).

This current study was conducted to determine the effect of the additional dietary NPP and vitamin D_3 on Ca and

Table 1: Ingredients and Calculated Nutrient Composition of Experimental Diets

NPP (%)	0		0.15		0.30		0.45	
Vitamin D IU/kg	0	3000	0	3000	0	3000	0	3000
Ingredients /groups	T-0	T-1	T-2	T-3	T-4	T-5	T-6	T-7
Corn	36.5	36.5	36.5	36.5	36.5	36.5	36.5	36.5
Soybean meal	12	12	12	12	12	12	12	12
Wheat	15	15	15	15	15	15	15	15
Barley	12	12	12	12	12	12	12	12
Meat bone meal	2.14	2.14	2.14	2.14	2.14	2.14	2.14	2.14
Sunflower meal	5.97	5.97	5.97	5.97	5.97	5.97	5.97	5.97
Ful fat soybean	5	5	5	5	5	5	5	5
Narble meal	9.1	9.1	9.1	9.1	9.1	9.1	9.1	9.1
Vitamin premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09
Methionin	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
Animal fat	1.34	1.34	1.34	1.34	1.34	1.34	1.34	1.34
Calculated Analysis								
Crude protein	16	16	16	16 16	16	16	16	
ME Kcal/kg	2650	2650	2650	2650	2650	2650	2650	2650
Ca %	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75
Total P %	0.5	0.5	0.65	0.65	0.80	0.80	0.95	0.95

P levels of serum and eggshell in laying hens in late egg production period.

Materials and Methods

One hundred ninety-two 70 weeks old white Lohman LSL laying hens were used in this experiment. Birds were randomly assigned to eight groups equally (n = 24) and housed environmentally controlled laying house, each treatment was replicated six times, four birds were settled down per cage (50 x 46 x 46 cm). Groups were equally distributed in the upper and lower cage levels to minimize cage-level effect.

There were eight dietary treatments (one control and seven experimental groups). Control birds were fed on a basal diet containing about 16 % CP, 2650 kcal ME/kg (Table 1). Experimental diets were prepared by adding inorganic phosphorous at the levels of 0, 0.15 0.30 and 0.45% to basal diet with and without vitamin D_3 (3000 IU/kg) respectively (T0, T1, T2, T3, T4, T5, T6 and T7). Feed and water were supplied for *ad libitum* consumption. Also hens were exposed to 16 h of light/day. And this study lasted for 8 weeks feeding period, blood samples were obtained from the brachial veins of six hens per treatment, the serum was separated by centrifugation blood for 10 min. at 200 x g and saved for determination of serum Ca and P. The Ca and P were measured on auto analyzer by using commercial kits. Eggshell Ca and P contents were analyzed by atomic absorption spectrophotometer (AAS).

Statistical analyses was performed by the statistical package SPSS (1999) for windows, version 10.0. Multiple comparison of the data was done by using the Duncan test after one-way analysis of variance (one-way ANOVA).

Results and Discussion

This experiment was conducted to test the effect of dietary nonphytate phosphorus level and vitamin D_3 on plasma calcium and phosphorus levels, eggshell calcium and phosphorus levels of laying hens in late production periods.

In this study all parameters were measured these the plasma Ca , plasma P, eggshell Ca and eggshell P levels were affected by dietary nonphytate phosphorus levels and vitamin D_3 (Table 2) by statistically significant ($P<0.01$). Nonphytate phosphorus supplementation decreased plasma calcium levels inversely, increased eggshell calcium concentration. NPP supplementation also increased both plasma and eggshell P levels, linearly as increasing dietary NPP level when vitamin D_3 was omitted from diet. These results agree with those of Miles *et al*. (1983); Keshevarz (1994); Choi *et al*. (1979); Lim *et al*. (2003); Boorman and Gunaratne (2001) and Fros *et al*. (1991).

Vitamin D_3 supplementation decreased plasma Ca level whereas, increased eggshell Ca level linearly as increasing dietary NPP level except, the group (T1) fed diet with vitamin D_3 and without NPP supplementation. Vitamin D_3 supplementation also decreased plasma P level as increasing dietary NPP level, whereas increased eggshell P level in five groups (up to 0.30% NPP supplementation) linearly.

Plasma Ca level of the T1 (unsupplemented NPP but with vitamin D_3) was significantly greather than those fed the other diets. However, T7 with 0.45% of the high

Table 2: Effect of dietary nonphytate phosphorus level on Ca and P level of serum (mg/dl) and eggshell (%)

Groups	Calcium		Phosphorus	
	Serum	Eggshell	Serum	Eggshell
T-0	9.7[ab]	16.49[c]	4.60	0.105[e]
T-1	10.05[a]	19.39[a]	4.60	0.147[b]
T-2	9.87[a]	17.13[b]	5.05	0.105[b]
T-3	9.52[ab]	19.89[a]	4.85	0.167[a]
T-4	9.22[bc]	17.30[b]	7.22	0.147[b]
T-5	8.90[c]	15.12[d]	6.57	0.126[c]
T-6	7.90[d]	14.44[e]	8.42	0.115[d]
T-7	7.95[d]	16.63[c]	7.87	0.113[e]
SEM	0.062	0.632	0.114	0.010
P	**	**	**	**

**: p< 0.01

supplemental NPP and without vitamin D_3 had lower plasma Ca than others. The highest eggshell Ca content rations was obtained from hens (T3) fed the diet supplementing 0.15% NPP and with vitamin D_3. whereas, the lowest eggshell Ca concentration was obtained hens (T7) fed diet containing high level of supplemental NPP without vitamin D_3. The plasma P level of T7 was significantly greater than those fed the other diets. However, the lower plasma P level was obtained from hens fed control diet. (T0). Vitamin D_3 supplementation increased both Ca and p levels in eggshell at diet (T3) with supplemental 0.15% NPP.

The objectives of the composition of the egg shell of poultry fed with diets containing different level of nonphytate and vitamin D_3 and to provide basic information for the eggshell calcium and phosphorus of hens since, no report was found on this respects in the literature to compare of this dates.

It is also considered that the differences of calcium and phosphorus levels in both plasma and eggshell is due to increased calcium excretion which at least in part, is mediated by academia, resulting from the dietary changes of acid-base balance. However our data has not sufficient to establish a direct link between academia and calcium excretion.

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