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## The Effects of Calcium and Vitamin D<sub>3</sub> in Diet on Plasma Calcium and Phosphorus, Eggshell Calcium and Phosphorus Levels of Laying Hens in Late Laying Production Period

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**Abstract:** This experiment was carried out to investigate the effect dietary calcium level and vitamin-D<sub>3</sub> on calcium and phosphorus concentrations in plasma and eggshell of laying hens in late production period. Two hundred forty 70 weeks white lohman LSL laying hens were randomly assigned to ten groups equally (n=24) each treatment was replicates six times. Experimental diets were prepared by adding calcium at the levels of 0, 1, 1.5, 2, and 2.5% to basal diet with and without vitamin D<sub>3</sub> (3000 IU/kg) respectively (T1 e.t T10). All diets were isocaloric and isonitrogenous, study was lasted eight weeks. Plasma Ca and P, eggshell Ca and P concentrations were evaluated in this study. All parameters were measured these plasma Ca and P, eggshell Ca and P levels were affected from dietary treatments by statistically significant (P< 0.01). Calcium supplementation significantly (P<0.01) increased plasma Ca level linearly, as increasing dietary Ca level. Ca supplementation also caused significant differences both Ca and P levels of eggshell and plasma P concentrations. Eggshell Ca concentration of the T5 (supplementing 1.5% Ca) was significantly greater than those fed the others diets. However the highest eggshell P level was in T7 (adding 2 % Ca), regardless of vitamin D<sub>3</sub> in diet. As for vitamin D<sub>3</sub> supplementation significantly (P<0.01) increased Ca concentration both plasma and eggshell. Vitamin D<sub>3</sub> supplementation also increased P concentration in plasma.

**Key words:** Laying hen, vitamin D<sub>3</sub>, dietary Ca, eggshell Ca, serum Ca

### Introduction

The influence of various factors on shell quality have been revived by a number of investigators (Wasburn, 1982; Zapato and Gernat, 1995; Camerius *et al.*, 1996). Genetics, age, nutrition and environment are the major factors that influence eggshell quality (Al-Batshan *et al.*, 1994) calcium is one of the key element required for maintenance and production of laying hens. It is the most abundant inorganic component of the skeleton and plays a major role in the wide variety of biological function (Mahmoud *et al.*, 1994). Comprising about 1.5% of a birds weight, calcium is the predominant mineral in body. Calcium is used for bone formation, eggshell production and blood clotting. It also affects the heard, muscles and nerves as well as some of the body's enzyme systems. Most of the body's Ca is found in the skeleton, calcium is comprised mainly of calcium phosphate with some calcium carbonate. Calcium carbonate is the main compound found in egg shells (Highfill, 1998). It is generally accepted that the decrease in egg production rates and increase in egg size, without concurrent and equal increase in shell weight is the reason for the decrease in shell quality as the hen ages (Ousterhout, 1980; Lee, 1982; Summers and Leeson, 1983; Al-Bustany and Elwinger, 1987). Eggshell quality also change in accordance with level of production and age of layer. As age advances also, shell thickness and shell strength decrease as age advances (Camerius *et*

*al.*, 1996). Calcium is major macro-mineral involving shell formation is increased with shell weight resulting from increase egg weight. However, the hen's ability to absorb from the digestive system and to mobilize Ca from the medullar bones (Due to depletion of Ca of these bones) is reduced with age (Keshavarz and Nakajima, 1993). Among the various nutritional factors that are necessary for proper eggshell formation adequacy of Ca intake probably, plays the important role, because Ca make up about 40% of the eggshell investigation of the Ca requirement of laying hens have been the subject of numerous reports. The shell deposition and shell quality are directly related to the calcium level in the diet.

The value of 3.75 g Ca per hen per day or greater was determined to be necessary for optimum shell and bone formation (Clunies *et al.*, 1992). However, Roush *et al.* (1986) reported 4.73% Ca was the optimum level for shell formation in laying hens.

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-Dihydroxycholecalciferol [1,25 (OH)<sub>2</sub> D<sub>3</sub>] on intestinal function (Vaiano *et al.*, 1994). It is well established that in laying hens a vitamin D<sub>3</sub> dependent Ca-binding protein is involved in the active transport of Ca across the intestinal membrane and probably across the uterine membrane. Dietary and

Table 1: Ingredients and Calculated Nutrient Composition of Experimental Diets

Ca %	0		1		1.5		2		2.5	
Vitamin D IU/kg	0		3000		0		3000		0	
Ingredients /groups	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
Corn	36.5	36.5	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	12	12
Wheat	15	15	15	15	15	15	15	15	15	15
Barley	12	12	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	2.14	2.14
Sunflower meal	5.97	5.97	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	5	5
Narble meal	9.1	9.1	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	0.25	0.25
Salt	0.25	0.25	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	0.09	0.09
Methionine	0.08	0.08	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	1.34	1.34
Calculated Analysis										
Crude protein	16	16	16	16	16	16	16	16	16	16
ME Kcal/kg	2650	2650	2650	2650	2650	2650	2650	2650	2650	2650
Total Ca %	3.5	3.5	3.90	3.90	4.10	4.10	4.30	4.30	4.50	4.50
P %	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

endogenous vitamin D<sub>3</sub> is first hydroxylated position 25 of the vitamin D<sub>3</sub> molecule in the liver to produce 25-OH cholecalciferol (25-OH-D<sub>3</sub>) which is the main circulating vitamin D<sub>3</sub> metabolite in the blood. The circulating 25-OH-D<sub>3</sub> is then further hydroxylated in position of the molecule in the kidney to produce 1,25 (OH)<sub>2</sub> D<sub>3</sub>. this active form of vitamin D<sub>3</sub> 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, Vaiano *et al.*, 1994, Keshavarz, 2003). 1,25 (OH)<sub>2</sub>D<sub>3</sub> also influence alkaline phosphates activity in duodenal mucosa (Grunder and Tsang, 1990).

This current study was conducted to determine the effect of the additional dietary Ca and vitamin D<sub>3</sub> on Ca and P levels of serum and eggshell in laying hens in late egg production period.

### Materials and Methods

Two hundred forty 70 weeks old white Lohman LSL laying hens were used in this experiment. Bird were randomly assigned to ten groups equally (n=24), each treatment was replicated six times and housed environmentally controlled laying house, each treatment was replicated six times, four hens 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 ten dietary treatments (one control and nine experimental groups). Control birds were fed on a basal diet (T1) containing about 16 % CP, 2650 kcal ME/kg and 3.5% Ca (Table 1). Experimental diets were prepared by adding limestone (containing about 40% Ca) at the

levels of 1, 1.5, 2 and 2.5% to basal diet with and without vitamin D<sub>3</sub> (3000 IU/kg) respectively (T2, T3, T4, T5, T6, T7, T8, T9 and T10). This limestone addition provided Ca to basal diet at the levels of about 0.4, 0.6, 0.8 and 1% respectively. All diets were isocaloric and isonitrogenous. Experiment was lasted eight weeks. Feed and water were supplied for *ad libitum* consumption. Also hens were exposed to 16 h of light/day. At the end of the eight weeks feeding period, blood samples were obtained from the brachial veins of six hens per treatment, the plasma was separated by centrifugation blood for 10 min. at 2000 x g and saved for determination of plasma Ca and P. The Ca and P were measured on auto analyzer by using commercial kits. Eggshell samples were collected from four hens per experiment at the end of the experiment. Eggshell Ca and P concentrations were analyzed by atomic absorption spectrophotometer (AAS).

Statistical analyses was performed by the statistical package SPSS (1999) for windows, version 10.0. Multiple comparisons of the data were 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 calcium level and vitamin D<sub>3</sub> on plasma calcium and phosphorus levels, eggshell calcium and phosphorus levels of laying hens in late production period.

In this study all parameters were measured these the plasma Ca , plasma P, eggshell Ca and eggshell P levels were affected by Ca levels and vitamin D<sub>3</sub> (Table 2) by statistically significant (P<0.01). Calcium

Table 2: Effect of dietary Ca levels on Ca and P levels of plasma and eggshell

Groups	Calcium		Phosphorus	
	Plasma	Eggshell	Plasma	Eggshell
T-1	9.7 <sup>e</sup>	16.49 <sup>b</sup>	4.60 <sup>ab</sup>	0.105 <sup>c</sup>
T-2	10.05 <sup>e</sup>	19.39 <sup>a</sup>	4.60 <sup>ab</sup>	0.147 <sup>a</sup>
T-3	12.12 <sup>d</sup>	14.08 <sup>d</sup>	4.95 <sup>a</sup>	0.075 <sup>e</sup>
T-4	12.20 <sup>d</sup>	15.89 <sup>a</sup>	4.98 <sup>a</sup>	0.115 <sup>bc</sup>
T-5	14.55 <sup>c</sup>	16.88 <sup>b</sup>	3.35 <sup>f</sup>	0.110 <sup>bc</sup>
T-6	13.18 <sup>d</sup>	17.65 <sup>ab</sup>	4.22 <sup>bcd</sup>	0.095 <sup>d</sup>
T-7	15.72 <sup>bc</sup>	15.10 <sup>c</sup>	3.62 <sup>ef</sup>	0.120 <sup>b</sup>
T-8	15.75 <sup>bc</sup>	16.60 <sup>b</sup>	4.30 <sup>abcd</sup>	0.115 <sup>bc</sup>
T-9	17.07 <sup>a</sup>	12.09 <sup>e</sup>	3.78 <sup>def</sup>	0.115 <sup>bc</sup>
T-10	16.20 <sup>ab</sup>	13.98 <sup>d</sup>	4.45 <sup>abc</sup>	0.110 <sup>bc</sup>
SEM	1.038	1.032	1.072	0.003
P	**	**	**	**

\*\* : p < 0.01

supplementation increased plasma calcium levels linearly, decreased eggshell calcium concentration. Ca supplementation also decreased both plasma P level, increased eggshell P levels, linearly as increasing dietary Ca level when vitamin D<sub>3</sub> was omitted from diet. These results agree with those of Atteh and Leeson (1983); Clunies *et al.* (1992) and Keshavarz (1994). Vitamin D<sub>3</sub> supplementation increased plasma Ca level and eggshell Ca level linearly as increasing dietary Ca level except, the groups (T6 and T10). Vitamin D<sub>3</sub> supplementation also decreased plasma P level as increasing dietary Ca level, whereas increased eggshell P level in two groups (up to 1.5 % Ca supplementation) linearly.

Plasma Ca level of the T9 (without vitamin D<sub>3</sub>) was significantly greater than those fed the other diets. However, T1 (control) had lowest plasma Ca than others. The highest eggshell Ca level was obtained from hens (T2) fed the basal diet with vitamin D<sub>3</sub>. Whereas, the lowest eggshell Ca concentration was obtained from hens (T9) fed diet containing high level of supplemental Ca (2.5 %) without vitamin D<sub>3</sub>. The plasma P level of T3 and T4 were significantly greater than those fed the other diets. However, the lower plasma P level was obtained from hens fed 1.5 % Ca without vitamin D<sub>3</sub> (T5). Vitamin D<sub>3</sub> supplementation significantly (P < 0.01) increased Ca levels both plasma and eggshell. Vitamin D<sub>3</sub> supplementation also increased plasma P level.

The objectives of the composition of the egg shell of poultry fed with diets containing different level of Ca and vitamin D<sub>3</sub> 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.

In conclusion, the information obtained from this

experiment considered that vitamin D<sub>3</sub> supplementation increased calcium deposition in eggshell by increasing calcium absorption from intestinal system. It is also indicated that laying hens can tolerate relatively high dietary levels of calcium with vitamin D<sub>3</sub> without adverse effect on their health.

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