The Effects of Calcium and Vitamin D₃ 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-D₃ on calcium and phosphorus concentrations in plasma and eggshell of laying hens in late production period. Two hundred forty 70 weeks white lohan USL 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 D₃ (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 D₃ in diet. As for vitamin D₃ supplementaion significantly (P<0.01) increased Ca concentration both plasma and eggshell. Vitamin D₃ supplementation also increased P concentration in plasma.

Key words: Laying hen, vitamin D₃, 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 Gernart, 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 bird’s weight, calcium is the predominant mineral in body. Calcium is used for bone formation, eggshell production and blood clotting. It also affects the heart, 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-Dihydroxycalciferol (1,25 (OH)₂ D₃) on intestinal function (Vaiano et al., 1994). It is well established that in laying hens a vitamin D₃ dependent Ca-biding protein is involved in the active transport of Ca across the intestinal membrane and probably across the uterine membrane. Dietary and
endogenous vitamin D₃ is first hydroxylated position 25 of the vitamin D₃ molecule in the liver to produce 25-OH cholecalciferol (25-OH-D₃) which is the main circulating vitamin D₃ metabolite in the blood. The circulating 25-OH-D₃ is then further hydroxylated in position of the molecule in the kidney to produce 1,25 (OH)₂-D₃. This active form of vitamin D₃ 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)₂-D₃ 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 D₃ 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 D₃ (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 (1989) 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 D₃ 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 D₃ (Table 2) by statistically significant (P<0.01). Calcium
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 D₃ was omitted from diet. These results agree with those of Atteh and Leeson (1983); Clunies et al. (1992) and Keshavarz (1994). Vitamin D₃ supplementation increased plasma Ca level and eggshell Ca level linearly as increasing dietary Ca level except, the groups (T6 and T10). Vitamin D₃ 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 D₃) 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 D₃. Whereas, the lowest eggshell Ca concentration was obtained was hens (T9) fed diet containing high level of supplemental Ca (2.5 %) without vitamin D₃. 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 D₃ (T5). Vitamin D₃ supplementation significantly (P<0.01) increased Ca levels both plasma and eggshell. Vitamin D₃ 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 D₃ 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 D₃ 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 D₃ without adverse effect on their health.

References

SPSS, 1999. SPSS For Windows Release 10.0, SPSS Inc.


