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Assessing Bone Mineral Density, Eggshell Characteristics and their Relationship at Peak Egg Production of Laying Hens in Response to Various Levels of Vitamin C

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ABSTRACT

In this study, the effects of the inclusion of different vitamin C levels in the diet on bone mineral density (BMD) and eggshell quality was determined. Four experimental diets with four levels of vitamin C (0, 250, 500 and 750 mg/kg) were used. During the experiment, environmental temperature and humidity were 19±1°C and 55±5% respectively. Correlation coefficients of BMD index and shell characteristics with production parameters were also determined. Egg weight, egg mass, hen-day egg production and eggshell quality were not significantly influenced by vitamin C levels. BMD was not affected by vitamin C utilization. A positive correlation was observed between BMD and egg weight and hen-day egg production. A significant positive correlation between specific gravity and eggshell traits was also found. In conclusion, the results of this study have shown that vitamin C does not affect bone mineral density and eggshell quality under lower critical temperature.

INTRODUCTION

It is quite normal for high-producing hens to use the calcium reserves in their bones to produce eggshells, and hens use calcium from the medullary bone by a process of resorption (Abdul-Aziz, 1998). Vitamin C may play a role in improving bone properties (Orban *et al.*, 1993), which may be ascribed to increased calcium absorption, where vitamin C could play a role in the development of bone tissue.

There is considerable evidence that vitamin C is essential for type I collagen matrix synthesis, alkaline phosphatase activity, osteocalcin accumulation and matrix mineralization in bones (Franceschi *et al.*, 1993; Newman & Leeson, 1997). Vitamin C is also involved in vitamin D_3 metabolism. In the liver, vitamin D_3 is converted into 25-hydroxy cholecalciferol, and in the kidney, it is changed to 1,25-di-hydroxy cholecalciferol by 1-α-hydroxylase, which is activated by vitamin C. 1,25-di-hydroxy cholecalciferol can initiate Ca mobilization from Ca reserves of bones for eggshell production if necessary (Abe *et al.*, 1982).

An *in-vivo* study with broiler chicks (Weiser *et al.*, 1992) showed that vitamin C supplementation (200 mg/kg diet) improved bone weight, ash and hydroxyl proline contents and bone breaking strength by synergistic effects of dietary vitamin D_3 and vitamin C supplements (Franchini *et al.*, 1993). Opposite findings were reported:vitamin C had no beneficial effect on bone characteristics (Keshavarz, 1996) or little response (Newman & Lesson, 1999).

Recent studies have evaluated the effects of vitamin C supplementation on eggshell quality. Some of the studies showed that supplemental vitamin C was not able to affect eggshell quality or Ca absorption and reduced excretion in the kidney, the only alternative source of this mineral is the skeleton. Poor eggshell quality is associated with an increase in egg size and lower intestinal mineral absorption of minerals by birds (Leeson & Summers, 2000). Although poultry are able to synthesize vitamin C, and therefore it does not need to be supplemented in layer diets under not stressful and normal conditions, but the role of vitamin C in Ca retention in bones used as Ca storage for eggshell formation should be determined. Therefore, the present study aimed at determining the effect of vitamin C supplementation on BMD and some eggshell characteristics under rearing conditions in Iran.

MATERIAL AND METHODS

Housing and birds

A total of 190, 20-week-old Hi-Line® 36 layer hens were housed in 64 cages (42 cm×50 cm×40 cm). Each treatment included four replicates of four cages with three birds each. Hens were maintained under a 16 h light- 8 h dark schedule.

The control diet was a commercial diet containing 180 g/kg crude protein (CP) and 11.93 MJ AMEn/kg. Vitamin C was added to the control diet in increments of 250 mg to the other three diets up to 750 mg vitamin C. Feed and water were offered *ad libitum*. The poultry house was lit for 16 hour per day. During the experiment, house temperature (19±1°C) and humidity (55±5%) were recorded. The experiment lasted for 105 days. The number of eggs and egg weight were daily recorded throughout the experiment. Egg mass was calculated by multiplying egg production percentage by egg weight for each replicate. Egg production was expressed by hen-day egg production.

Bone mineral density index and eggshell parameters

Bone mineral density (BMD) index was determined in two hens per replicate in the right metatarsus bone by the radiographic imaging at the end of experiment as described by Hester *et al*. (2004). An electronic sensor placed under the metatarsus received the rays. Images were digitalized in a computer. The standardized region for reading was the middle of right metatarsus. Radiographic optical density readings (bone mineral density index) were carried out using the software Planmeca Dimaxis Classic 3.2.2, and all readings followed the same pattern. This software

points. Light points indicate high BMD index, whereas BMD index is lower where points are darker.

For eggshell quality analysis, 25 eggs were randomly collected per treatment. Specific gravity was calculated (Equation 1) and eggshell percentage was determined by dividing eggshell weight to egg weight.

Specific gravity= W/ (0.968 W - 0.4759 SW) Eq. 1
(Kul & Seker, 2004)

Where:

W= Egg weight (g); ESW= Eggshell weight (g)

Chemical analysis

Eggshell Ca and P contents were determined as follows: eggshells were collected and dried in an oven at 60°C for 48 h and ground for chemical analysis. Organic matter of the samples was removed by hydrochloride acid. Ca and P contents of the eggshells were measured by ICP[1] (GBC Integra XL). Mineral assays were calibrated using a series of mixtures containing graded concentrations of standard solutions.

Experimental design and statistical analysis

The experiment was conducted as a completely randomized design (CRD). General linear models were employed for statistical analysis. Analysis of variance and polynomial contrasts were conducted using Proc GLM of SAS (2004). Significant differences between treatments were determined using Duncan's multiple range test at p≤0.05. Correlations between these characteristics and bone mineral density (BMD) index were calculated using the test of Pearson.

RESULTS

Vitamin C supplementation had no effect (p>0.05) on egg weight, egg mass and hen-day egg production (Table 1). The BMD index was not affected (p>0.05) by vitamin C supplementation (Table 1). Eggshell characteristics (thickness, weight, percentage, Ca and P content) were not significant difference by vitamin C (p>0.05).

A high correlation was observed between eggshell characteristics and specific gravity (Table 2). Specific gravity was highly correlated with eggshell thickness and eggshell weight (r = 0.84 and r = 0.60, respectively, p<0.01). Egg weight was positively correlated with

Table 1 - Effect of vitamin C levels (0, 250, 500 and 750 mg/kg diet) on egg production parameters, BMD[1] index and eggshell characteristics.

	Treatment (vitamin C level)						P-value of Polynomial contrast		
	Control	250 mg	500 mg	750 mg	*P-value*	SEM	Linear	quadratic	cubic
Egg weight (g/egg)	58.19	58.56	57.99	57.65	0.74	0.413	0.427	0.650	0.570
Hen - day egg production	92.56	93.15	89.28	92.85	0.31	1.178	0.949	0.578	0.375
Egg mass (g/hen/day)	53.84	54.55	51.77	53.56	0.32	0.912	0.844	0.751	0.520
BMD	158.86	161.62	162.5	156.74	0.37	4.407	0.885	0.245	0.740
Eggshell thickness (mm)	0.355	0.357	0.350	0.350	0.15	0.002	0.084	0.722	0.142
Eggshell weight (g)	4.86	5.04	4.93	4.87	0.20	0.063	0.751	0.072	0.259
Eggshell percentage (%)	9.07	9.16	9.01	8.96	0.34	0.317	0.167	0.352	0.292
Specific gravity	1.0814	1.0806	1.0805	1.0815	0.28	0.002	0.165	0.355	0.289
Eggshell calcium (%)	31.20	32.14	34.62	32.34	0.81	2.510	0.609	0.533	0.586
Eggshell phosphorus (%)	0.16	0.15	0.16	0.12	0.93	0.054	0.632	0.762	0.806

Means with the same letter in each row are not significantly different at level of p<0.05. 1 - Bone mineral density.

eggshell weight ($r = 0.54$, $p<0.05$) and negatively correlated with eggshell percentage ($r = -0.38$, $p<0.01$). The correlation between hen-day egg production and eggshell characteristics was not significant ($p>0.05$).

Table 2 - Correlation coefficients between BMD[1] index and shell characteristics with production parameters.

	Egg weight	Hen-day egg production	Specific gravity
BMD index	0.54*	0.35*	0.11 NS
Eggshell weight	0.49 NS	0.01 NS	0.60**
Eggshell thickness	0.04 NS	0.02 NS	0.84**
Eggshell percentage	-0.38**	0.18 NS	0.99**

NS - Not significant. *p<0.05. **p<0.01. 1 - Bone mineral density.

DISCUSSION

In this study dietary vitamin C had no effect on BMD. It is seems that the unstressful conditions and early age of the birds were the main reasons of such result, because environmental stress has been shown to increase mineral excretion (Smith & Teeter, 1987; El-Husseiny & Creger, 1981). Keshavarz (1996) indicated that vitamin C supplementation had no beneficial effects on bone mineralization in laying hens. Newman & Lesson (1999) also noted that vitamin C generally had little effect on poultry bone characteristics. However, bone strength and ash content were improved with the supplementation of 200 mg vitamin C/kg diet (Wiser *et al*., 1992).

In the present study vitamin C had no effect on eggshell quality (eggshell thickness, eggshell weight, and eggshell percentage). This result is in agreement with the findings of Keshavarz (1996), Amaefule *et al*. (2004) and Ciftici *et al*. (2005), who did not find any effect of vitamin C on eggshell quality and eggshell temperature of the poultry house during the period of the experiment (under 28 °C), which was not critical for heat stress. Pardue, & Thaxton (1984) and Balnave & Zhang (1992) found that vitamin C prevented eggshell quality reductions. Under both normal and heat stress conditions, vitamin C improved egg weight (Kucuk *et al*., 2003, Keshavarz, 1996), but in other studies, no response to supplementation vitamin C was observed under stress (Puthpongsiriporn *et al*., 2001) or normal conditions (Balnave & Zhang, 1992; Amaefule *et al*., 2004).

In the current study, no significant correlation was found between eggshell quality traits and metatarsus BMD index. These findings indicate that laying hens did not use bone Ca and P for eggshell formation when dietary Ca and P levels were sufficient.

Egg production peak is an important stage of the production period of laying hens. The highest requirement of calcium for eggshell formation occurs at peak production (Roland, 1986). It is hypothesized that high egg production may severely affect bone mineral density. In the present study, the correlation between egg production and BMD index was not high, in agreement with Almeida Paz *et al*. (2006), who observed that there was no need of bone mineral mobilization during the production period. Specific gravity is an indirect mean of eggshell evaluation (Hammerle, 1969). There was positive correlation between specific gravity and eggshell characteristics (especially eggshell percentage) in the present experiment. This is in agreement with the results of North & Bell (1990) and Almeida Paz *et al*. (2006).

It was possible to concluded that vitamin C had no influence on bone mineral density and some eggshell characteristics under low critical temperature mineral density and no effect on eggshell quality at the peak of production as it presented low correlation with BMD index and no correlation with eggshell quality. Further investigations need to be carried out to determine the effect of vitamin C in very low ambient temperature on BMD and also egg quality.

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