



Effect of vitamin K on bone integrity and eggshell quality of white hen at the final phase of the laying cycle

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ABSTRACT - The effect of four levels of dietary vitamin K (vit. K) on production, egg quality and bone structure of laying hens near the end of the production cycle were studied. A total of 192 Hy-Line, W-36 hens, 67 weeks of age, were distributed into a completely randomized design with four treatments (0, 2, 8, 32 mg vit. K/kg of diet), six replicates and eight birds per experimental unit. Corn-soybean-meal basal diets were isonitrogenous (15.5% crude protein), isoenergetic (2,790 kcal ME/kg), isocalcium (4.25% Ca) and isophosphorus (0.40% available P). Vitamin K supplementation did not alter egg mass, feed intake, feed conversion (kg/kg), bone breaking strength, specific egg gravity, eggshell weight, thickness and percentage of thin and cracked shell. A linear effect on egg weight, laying percent, and feed conversion (kg/dozen) was observed, as well as a quadratic effect on the ash bone content. In conclusion, the inclusion of increasing levels of vitamin K to the diet influenced performance and bone mineralization, but not eggshell quality. The lack of consistency in the efficiency of supplemental vitamin K on eggshell quality may be due to the age of hens.

Key Words: eggshell quality, layer, osteoporosis

Efeito da vitamina K sobre a integridade óssea e da casca de ovos de poedeiras leves na fase final de postura

RESUMO - Objetivou-se com este estudo avaliar o efeito de quatro níveis dietéticos de vitamina K (vit. K) sobre o desempenho e a qualidade de ovos e ossos de poedeiras leves na fase final de postura. Foram utilizadas 192 poedeiras comerciais com 67 semanas de idade, distribuídas em delineamento casualizado, com quatro níveis de vitamina K (0, 2, 8, 32 mg/kg), seis repetições e oito aves por unidade experimental. As rações eram isoprotéicas (15,5% de proteína bruta), isoenergéticas (2.790 kcal de energia metabolizável/kg), isocalcicas (4,25% cálcio) e isofosfóricas (0,40% fósforo disponível) e foram formuladas à base de milho e farelo de soja. A suplementação de vitamina K não alterou a massa de ovos, o consumo de ração, a conversão alimentar (kg/kg), a resistência óssea a quebra, a gravidade específica do ovo, o peso da casca, o percentual de ovos de casca fina e quebradas e a espessura da casca. Houve efeito linear dos níveis de vitamina K sobre o peso do ovo, a taxa de postura e a conversão alimentar (kg/dz) e efeito quadrático sobre o teor de cinzas ósseas. A inclusão de vitamina K influenciou o desempenho produtivo e a mineralização óssea, mas não influenciou a qualidade da casca dos ovos. A ausência de resposta da qualidade da casca à suplementação de vitamina K pode estar relacionada à idade das poedeiras.

Palavras-chave: qualidade da casca, poedeiras, osteoporose

Introduction

The loss of structural mass of fully mineralized bones leading to a bone fragility and susceptibility to fracture is a frequent problem in commercial laying hen. As in humans, the osteoporosis etiology is multifactorial, involving nutritional, environmental (management and housing) and genetic factors (Whitehead & Fleming, 2000).

Genetic selections to high egg production and low body weight are among the main causes of osteoporosis emergences on modern strains of commercial laying hens. According to Fleming et al. (2003), these birds lay almost one egg per day during at least six months; consequently, they keep a high estrogen rate that accelerates structural bone re-absorption in this period.

By the end of the first production period, the earliest signals of loss of structural bone, elevation of bone

re-absorption, and decline of bone mineralization are apparent. At the same time, a progressive fall on the blood estrogen levels prevents the formation of medullar bone (Rennie et al., 1997), whose purpose is to provide a labile source of Ca for shell formation. Thus, osteoclasts resorb structural bone to mobilize Ca, which results in a decline in the structural bone of hens. The progressive loss of structural bone during the laying period weakens the skeleton and increases the risk of fracturing bones, characteristic of osteoporosis (Fleming et al., 1998; Fleming et al., 2006).

According to Gregory & Wilkins (1989) almost a third part of end-lay hens suffer from osteoporosis. Besides, a recent study shows that about 20% of these birds are affected by bone fractures (Budgell & Silversides, 2004). These numbers are not only an extension of the economic problem due to the occurrence of osteoporosis but they also reflect the painful process that affects the welfare of these birds.

Among the nutritional factors involved in the process, the size particle of Ca sources is apparently the most important. Large particles remain for longer time in the crop and gizzard, delaying the gut Ca absorption, and reducing the mobilization of medullar Ca if coincident with the eggshell formation period (Rao & Roland, 1990; Whitehead & Fleming, 2000; Fleming et al., 2003).

Currently, there is some evidence that vitamin K affects the Ca balance. Vermeer et al. (1996) reported that vitamin K is involved in blood coagulation and bone metabolism via carboxylation reaction in which glutamic acid residues (GLA) transform the γ -carboxyglutamic glutamic acid into blood coagulation factors and bone proteins (osteocalcin, matrix GLA protein, and S protein). Osteocalcin is a low-weight molecular protein with three GLA residues produced by osteoblasts during bone matrix formation (Mijares et al., 1998). As one of the most abundant noncollagenous proteins of the extracellular bone matrix, its dosage in the blood is an important seric marker of bone formation (Dôres et al., 2001). Low level of vitamin K induces the synthesis of non-carboxyated osteocalcin that presents poor affinity for hidroxiapatite, leading to a deficient bone mineralization.

Despite the microbial vitamin K synthesis in the gut, the required quantity exceeds the synthesis. Moreover, the nature of the large intestine revetment that impairs absorption and the small extension of this gut segment in birds could probably lead to higher vitamin K requirements for bone quality than for blood coagulation.

Previous studies have shown that vitamin K was involved in bone metabolism in individuals suffering from

osteoporosis. Low vitamin K levels in the blood and bone of elderly women with hip fracture was verified by Hodges et al. (1993). Knapen et al. (1989) compared osteocalcin serum concentration in both pre and postmenopausal women and detected less than 40% of serum carboxyated osteocalcin level in the latter group, condition associated with the lack of vitamin K. More recently, Booth et al. (2000) observed that an insufficient vitamin K intake was related to increased incidence of hip fracture, but no correlation was observed with low bone mineral densities. Although the existence of some limitations in these epidemiological studies, they all acknowledge the role of vitamin K in delaying bone mineralization losses of elderly individuals (Olson, 2000).

Studies on the effects of vitamin K on poultry bone quality are scarce. The high vitamin K supplementation level decreased tibia bone loss from 15 to 25-week-old laying hens (Fleming et al., 1998) and the dietary increment of supplementary vitamin K from 0.5 mg/kg to 8 mg/kg reduced the serum concentration of decarboxyated osteocalcin and improved the broiler bone mineralization (Zhang et al., 2003).

This experiment aimed to evaluate the effects of supplementing vitamin K to diet of light laying hen on performance, external egg quality and bone integrity.

Material and Methods

The study was performed at the poultry facility of the *Iguatemi* Experimental Farm - Maringá State University, Maringá, state of Paraná, Brazil. A total of one hundred and ninety-two 67-week-old HyLine W36 laying hens were housed on 24 galvanized cages (30 cm wide x 45 cm deep) divided into four sections with two birds each, totaling eight birds per cage. Four treatments and six replications of eight birds each was assigned to a completely randomized design. The adjustment period to experimental diets after allotment lasted 14 days, followed by evaluation of four laying cycles of 28 days each, totaling 16 experimental weeks. The treatments consisted of a basal diet supplemented with 2, 8 and 32 mg vit. K/kg of diet. Menadione (2-metil-1,4Naftoquinone), 43% of bioavailability, was used as source of vitamin K. Basal diet (Table 1) was formulated to meet nutritional requirements of 67-83-week-old light laying hens as proposed by Hy-Line breeder (Hy-Line W36, 2000), altering the vitamin K levels only. Birds were submitted to artificial lighting at sunset, in a 17-hour light program per day. Feed and water were supplied *ad libitum*.

Table 1 - Composition of basal diet

Ingredient	%
Corn	62.22
Soybean meal (45%)	23.45
Soybean oil	1.44
Salt	0.342
Dicalcium phosphate	1.46
Limestone	10.54
DL-Methionine	0.147
Vitamin-mineral premix ¹	0.40
Nutritional (calculated values)	
Crude protein (%)	15.50
ME (kcal/kg)	2790
Methionine + cystine (%)	0.61
Lysine (%)	0.86
Calcium (%)	4.25
Available phosphorus (%)	0.40

¹ Per kg of product: vit. A - 2500000 UI; vit. D3 - 500000 UI; vit. E - 3750 mg; vit. B1 - 375 mg; vit. B2 - 1.000 mg; vit. B6 - 250 mg; vit. B12 - 2.500 mcg; calcium pantotenate - 3.750 mg; niacin - 7.500 mg; folic acid - 75 mg; Se - 62.5 mg; Mn - 18.750 mg; Fe - 25.000 mg; Cu - 1.000 mg; Zn - 125.000 mg; I - 150 mg.

Feed intake and conversion (kg ration/dozen eggs and kg ration/kg egg) data were recorded every 28 days. Egg production and number of eggs with thin or cracked shells were daily recorded. At the end of each laying period, the total egg production and egg production percent were calculated for each experimental unit.

At the end of every 28-day laying cycle, and for three consecutive days, egg quality, specific gravity (g/ml), shell yield (%), and shell thickness (mm) were analyzed. All eggs from each replication were individually identified and weighed on a precision digital scale (0.01g precision), and later submitted to a specific gravity test by flotation method in saline solution. Six solutions ranging in density from 1.070 to 1.090 with increments of 0.004 units were used. Specific gravities were checked by a petroleum densimeter. Washed and dried (48h at room temperature) shells from three eggs per replicate were weighed on a precision digital scale. Shell yield were obtained through the relation between egg weight and dry shell weight. Shell thickness was taken in four equatorial regions of each shell with the aid of a manual micrometer (Mitutoyo®).

At the end of experimental period, three birds/treatments were euthanized and their right tibias were collected to be used for bone breaking strength evaluation and bone ash percent. After being cleaned from muscle and fat, the tibias were boiled for 10 minutes, dried in stove (48 h at 105°C), and then submitted to a flexion assay at constant deformation force for viscoelastic materials in a manual press of simple measure with a 50-kg dynamometric ring, number 387, attached to a comparative Kofer apparatus at

0.001 mm of sensibility. The same bones were later defatted by successive immersion in petroleum ether for 8h, dried for 12h in stove at 100-103°C, weighed, ground and finally calcinated in a muffle at 900°C for bone ash content determination (AOAC, 1980).

Data were submitted to ANOVA test and the degrees of freedom relating to the vitamin K levels were decomposed in their regression components through orthogonal polynomial method using the SAEG procedure – Genetic Statistics Analysis System (UFV, 2001).

Results and Discussion

There were (Table 2) positive linear effects ($P < 0.05$) on egg production and feed conversion (kg/dozen). In addition, vitamin K supplemented diet affected ($P < 0.05$) the egg weight in a decreasing form (Table 3). Moreover, the obtained values are within the same category of “type 1 eggs, up to 60 g” from the official Brazilian egg classification, according to Resolution CIPOA n°5/91 (MAPA, 2006).

Vitamin K did not influence any external egg characteristic. However, the bone ash content showed a quadratic increase in function of the vitamin K levels ($P < 0.05$) (Table 3). This indicates that supplementation of 17.5mg/kg of vit. K was required to achieve good bone mineralization. Based on blood coagulation criterion, NRC (1994) recommends a supplementation of 0.5 mg/kg of vit. K, level herein considered inferior to maximum ash deposition.

Significant reduction in structural bone losses were observed near the end of the laying period of hens from 15 to 25 weeks of age supplemented with high vitamin K levels (Fleming et al., 1998). Accordingly, Zhang et al. (2003) found an improvement on bone quality of broilers fed higher vitamin K supplementation, above recommended levels (NRC, 1994). However, these researchers observed higher vitamin K requirements for optimum bone metabolism at the initial phase, and recommended supplementation of 8 and 2 mg of vit. K/kg to meet broiler requirements during starter and grower periods, respectively.

Vitamin K interferes with bone quality through its action on the osteocalcin carboxylation, improving the protein-hydroxyapatite bound capacity (Vermeer et al., 1996) Some studies (Binkey et al., 1995; Vermeer et al., 1996; Booth, 1997) have also reported a modulating effect of carboxylated osteocalcin on bone matrix mineralization.

Vitamin K supplementation can be more efficient during the early skeleton development. One priority of pullet

Table 2 - Performance and bone and shell quality of light laying hen at the final production phase

Characteristic	Supplementary vitamin K (mg/kg)				CV (%)	Effect*
	0	2.0	8.0	32		
Performance						
Egg weight, g	68.68	68.36	68.03	67.71	1.86	Linear
Egg production, %	74.52	75.61	76.70	77.79	6.64	Linear
Egg mass, %	51.77	51.12	52.02	52.40	6.80	ns
Feed intake, g	105.43	105.94	106.50	105.34	3.76	ns
Feed conversion, kg/dozen	1.710	1.686	1.661	1.637	6.25	Linear
Feed conversion, kg/kg	2.218	2.083	2.054	2.013	6.31	ns
Bone quality						
Bone breaking strength, kgf	82	86	84	82	14.30	ns
Bone ash, %	44.62	47.49	54.02	48.83	4.60	Quadratic
Eggshell quality						
Specific gravity, g/ml	1.076	1.076	1.076	1.077	0.15	ns
Shell yield, %	8.07	8.07	8.00	8.18	3.12	ns
Thin and cracked shell, %	2.46	1.52	2.53	1.59	39.50	ns
Shell thickness, mm	0.327	0.329	0.325	0.329	12.67	ns

* P<0.05; ns: not significant.

Table 3 - Regression equations of the characteristics

Characteristic	Regression	R ² (%)
Egg weight	$\hat{Y} = 69.0032 - 0.322987x$	79
Egg production	$\hat{Y} = 73.4251 + 1.09127x$	96
Feed conversion	$\hat{Y} = 1.73443 - 0.0243942x$	87
Bone ash	$\hat{Y} = 44.6218 + 1.52186x - 0.0434501x^2$	79

nutrition must be the formation of medullar bone (Whitehead & Fleming, 2000), assuring appropriate bone calcium supplies in the early laying period, crucial for the occurrence of osteoporosis reduction and for the maintenance of the production of eggs with good shell quality (Rao & Roland, 1990; Whitehead & Fleming, 2000).

During the starter laying period, a negative Ca balance occurs, and extra vitamin K supplementation may contribute to raise carboxylated osteocalcin, which can be bind to Ca from the bone matrix. This mechanism could result in a better Ca use, since the levels adopted by the poultry industry are already too high. Also, excessive Ca can impair the bioavailability of other minerals such as phosphorus, magnesium, manganese and zinc, and diet can become less palatable and dilute other components when high calcium carbonate levels (limestone) are used. The combined K and D vitamins supplementation could also improve the bone status of birds, maintaining the bone integrity of commercial laying hen, as observed in women (Douglas et al., 1995).

Conclusions

This study indicated that dietary vitamin K supplementation for light hens at the end of the laying phase influences the performance and bone mineralization, without any influence on the eggshell quality. The lack of consistency in the efficiency of supplementary vitamin K on eggshell quality can be due to the age of hens. Thus, it could be theorized that if vitamin K supplementation was performed on the pre-laying period, when the medullar bone is formed, more consistent results could have been obtained at final phase of the laying cycle.

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