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Effect of supplementation of diets for quails with vitamins A, D and E on performance of the birds and quality and enrichment of eggs[1]

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ABSTRACT - The objective of this research was to evaluate the performance of birds and the quality and enrichment of eggs from quails fed diets supplemented with vitamins A, D and E. Three experiments were performed, one for each vitamin, under completely randomized experimental design, with six replicates and eight birds per plot, totaling 192 quails. Performance of birds was evaluated by the daily feed intake, egg weight, laying percentage (%) and food conversion, per kg and dozen of eggs. It was also evaluated the internal quality (Haugh unit, yolk index and yolk and albumen percentages) and external quality (eggshell percentage, egg specific gravity, eggshell thickness and weight) and the concentration of vitamins in egg yolk by using the high performance liquid chromatography method. Vitamins supplementation did not improve productive performance neither the internal and external quality of the eggs, except for vitamin D supplementation, which increased intake. Incorporation of vitamin A in yolk increased 536.27% at level 30,000 UI/kg, vitamin D increased 13.43% at 1,500 UI/kg and vitamin E increased 479.05% at 600 UI/kg, and these results evidence that the nutritional value of eggs, related to vitamins, can be increased through supplementation of diets for quails.

Key Words: cholecalciferol, *Coturnix corturnix japonica*, retinol, tocopherol

Efeito da suplementação de dieta de codornas com vitaminas A, D e E sobre o desempenho das aves e a qualidade e o enriquecimento dos ovos

RESUMO - Objetivou-se nesta pesquisa avaliar o desempenho das aves e a qualidade e o enriquecimento de ovos de codornas sob suplementação com vitaminas A, D e E. Foram realizados três experimentos, um para cada vitamina, em delineamentos inteiramente casualizados, com seis repetições e oito aves por parcela, totalizando 192 codornas. O desempenho das aves foi avaliado pelo consumo diário de ração, peso dos ovos, porcentagem de postura e conversão alimentar, por kg e dúzia de ovos. Também foram avaliadas a qualidade interna (unidade Haugh, índice gema e porcentagens de gema e albúmen) e externa (porcentagem de casca, peso específico, espessura e peso da casca) e a concentração das vitaminas na gema dos ovos, mediante o método de cromatografia líquida de alta eficiência. A suplementação das vitaminas não melhorou o desempenho produtivo nem a qualidade interna e externa dos ovos, com exceção da suplementação de vitamina D, que elevou o consumo. A incorporação de vitamina A na gema aumentou 536,27% no nível de 30.000 UI/kg, a de vitamina D 13,43% no nível de 1.500 UI/kg e a de vitamina E 479,05% no nível de 600 UI/kg, e isso evidencia que o valor nutricional dos ovos, relacionado à vitamina, pode ser aumentado com a suplementação na dieta das codornas.

Palavras-chave: colecalciferol, *Coturnix coturnix japonica*, retinol, tocoferol

Introduction

Eggs contain several nutrients such as proteins, lipids, vitamins and minerals, and also other substances with important biological functions like essential amino acids. Millward (2004) claimed that the egg protein is highly bioavailable and its nutritional quality is higher when compared to other feed.

According to Pita et al. (2004), consumers are becoming even more aware on the important relationship between diet and health, and this importance has stimulated researchers and feed industry to develop products enriched with nutrients that can promote beneficial effects to health.

Allied to every positive aspects of performance and to low investments, raising quails has been calling attention of producers, companies and researchers (Murakami &

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Garcia, 2007). In Brazil, this can be proved by analyzing data from IBGE (2009), which registered an effective number of quails in 1995 of approximately 3 million and, in 2009, that effective increased up to 8 million.

Squires & Naber (1993) observed increase of retinol level in yolks of eggs from chicken fed vitamin A supplementation at levels that were one, two or four times higher than the recommended by NRC. Investigating the effect of supplementation and the fastness of cholecalciferol transfer from laying hens diets to egg yolks, Mattila et al. (2003) verified that the egg yolk enrichment reached the highest values after 8-13 days of supplementation and that after 112 days of supplementation, the concentration of cholecalciferol decreased gradually.

According to Batista et al. (2007), vitamin E present positive effects, as for feed nutritional quality as for human health. Food industry uses this vitamin as a natural antioxidant, an effective strategy to increase the intake of this micronutrient.

Watanabe (1999) described a change on egg vitamin profile through vitamin supplementation of diets with levels that were above from those recommended for maximum performance, resulting in a feed of better quality for human consumption.

Thus, the objective of this work is to evaluate the effect of vitamins A, D and E supplementation of diets for quails at levels superior than the requirements on performance and internal and external quality of eggs, and also to verify the effect of that supplementation on the concentration of those vitamins in yolk seeking for the enhancement of the nutritional levels of eggs.

Material and Methods

Experiments were performed in the Aviário Experimental in Departamento de Zootecnia at Faculdade de Ciências Agrárias e Veterinária, FCAV/UNESP – Jaboticabal, São Paulo, in a conventional facility for quails.

The experiment with vitamin A was performed with 192 quails which were 70 days of age, beak trimmed, distributed in a completely randomized experimental design and submitted to four diets (control; 10,000; 20,000 and 30,000 UI of vitamin A/kg of feed), with six replicates and eight birds per plot. Birds from control group were supplied with basal diet composed by normal levels of vitamin A recommended to supply their needs (Table 1) according to the nutrient composition tables from Rostagno et al. (2005) and their nutritional requirements, according to recommendations of Murakami et al. (1993) and NRC (1994). Treatments consisted in supplying this basal diet supplemented with vitamin A at levels above the requirements previously mentioned.

Within the same scheme, other two experiments were performed, differing on diets and ages of birds. The experiment with vitamin D was performed by using 192 quails at 44 weeks of age, beak trimmed and submitted to four diets (control and supplementation with vitamin D at the levels 1,500; 1,000 or 1,500 UI/kg of feed). And the experiment with vitamin E was performed with other 192 quails at 70 days of age, beak trimmed and supplied with four diets (control and vitamin E at levels 200; 400; 60 UI/kg of feed), both with six replicates and eight birds per plot.

Analyzed performance traits were: daily feed intake, egg weight, egg production (%) and food conversion (intake/dozen and kg of eggs), as well as internal quality (Haugh unit, yolk index, yolk and albumen percentage) and external quality (egg specific gravity, eggshell percentage, thickness and weight) and the quantification of vitamins in the egg yolk.

Four 14-day cycles were performed and at the end of the fourth cycle (56 days), three eggs from each replicate were collected randomly. Eggs were weighted, and the yolk was weighted separately. After weighting, the three yolks from each replicate were mixed, assembling a yolk pool which was later frozen and analyzed in the Laboratório do Departamento de Tecnologia in FCAV – UNESP at Jaboticabal.

Haugh unit was obtained by the relationship between egg weight (g) and albumen height (mm) with the help of a table micrometer (AMES® - S6428), according to Card & Nesheim (1966): $UH = 100*\log(H + 7.57 - 1.7W^{0.37})$, where: H = albumen height, in millimeters and W = egg weight, in grams.

Table 1 - Composition of feed supplied to birds at laying phase

Ingredient	%
Corn	63.51
Soybean meal	27.62
Dicalcium phosphate	2.60
Limestone	4.87
Salt	0.40
DL-methionine (98%)	0.17
L-lysine (78%)	0.33
Mineral and vitamin supplementation[1]	0.50
Calculated nutritional composition	
Crude protein (%)	18.00
Metabolizable energy (kcal/kg)	2,800
Calcium (%)	2.50
Available phosphorous (%)	0.55
Total methionine + cystine(%)	0.76
Total lysine (%)	1.30

[1] Mineral and vitamins supplementation (composition/kg of feed): folic acid - 0.31 mg; biotin - 0.12 mg; choline - 300 mg; niacin - 12.37 mg; calcium pantohenate - 3.56 mg; vit. A - 7,812.5 UI; vit. B_1 - 1.85 mg; vit. B_{12} - 25 mcg; vit. B_2 - 4.25 mg; vit. B_6 - 1.23 mg; vit. D_3 – 3,125 UI; vit. E - 15.62 mg; vit. K - 1.22 mg; copper - 9.37 mg; iodine - 0.63 mg; manganese - 57.18 mg; selenium - 0.28 mg; zinc - 72.28 mg; antioxidant - 0.5 mg.

The yolk index was evaluated with height and width measurements of the yolk, with the help of a caliper (Professional®), and the ratio between these two measurements resulted in the yolk index, meaning, IG = AG/LG.

Yolk, albumen and eggshell percentages were obtained after the eggs were broken, and the weights from yolk and dried eggshell were measured. The albumen weight was calculated by the difference between egg weight and the weights of yolk and eggshell. The ratio between the constituents of eggs was calculated by using the entire egg weight as quotient.

Specific gravity was obtained by immersing the intact eggs produced in the last day of each cycle in buckets with different saline solutions, whose densities varied from 1.050 to 1.085 with 0.005 intervals.

Eggshell thickness was evaluated with three measurements at the equatorial region with the help of a 0.001-mm precision micrometer (Mitutoyo®).

Cholecalciferol, retinol and tocopherol concentrations in yolk were obtained by high performance liquid chromatography (CLAE/HPLC). The instruments used for that were a Visible-UV detector (model SPD-M10Avp, SHIMADZU, 284 nm), column HRC-ODS (4.6 mm × 25 cm), reversed phase. Mobile phase with methanol gradient: water (98:2 for 4.5 minutes), flux 1.0 mL/minute, performed in duplicate and estimated in ìg/g of the sample for vitamins A and D and in mg/g for vitamin E. Extraction of vitamins in yolk was performed according to methodology by Cherian et al. (1996a) with some modifications.

Statistical analysis of the results was performed by polynomial regression procedure employing SAS® software program (SAS 9.1, SAS Institute, Cary, North Carolina, USA). Data was submitted to homogeneity evaluation and the identified outliers were removed. In case of statistical significance, means were compared by the Tukey test (P>0.05).

Results and Discussion

Vitamin A supplementation did not result in statistical difference for daily feed intake, egg weight, egg production and food conversion (by kg and by dozen of eggs) of quails during the experimental period (Table 2).

Similar results with vitamin A supplementation were described by Lin et al. (2002) and Mendonça Júnior et al. (2002), who also did not verify any differences on feed intake of hens supplied with vitamin A supplementation. However, Mori et al. (2003) noticed an increase on feed intake while hens were supplied with vitamin A at the level 30,000 UI/kg of feed.

Results obtained in this study for egg weight were different than those found by Fu et al. (2000), who analyzed different levels of retinol supplementation in diets of quails and observed an increase in eggs weight. In another experiment with quails, Bárdoz et al. (1996) did not verify differences in eggs weight when supplying birds with retinol (50,000 UI/kg).

Values of egg production (Table 2) were in agreement to the ones found by Ramalho et al. (2008) in a study where diets for quails were supplemented with increasing levels of retinyl palmitate directly by oral via and significant differences for this trait were not find. Differently, Lin et al. (2002) supplemented diets of hens with 3,000 and 9,000 UI/kg and observed significant influence of the levels of retinyl palmitate on eggs production.

Vitamin A supplementation of feed in this work did not enhance food conversion (kg and dozen of eggs), agreeing with the results found by Mendonça Júnior et al. (2002) and Mori et al. (2003) in research with vitamin A supplementation in diets of laying hens. Different results were found by Reid et al. (1965), who observed an increase in the conversion indices of laying hens supplied with vitamin A supplementation in diet.

Internal quality of eggs, evaluated by Haugh unit, yolk index, albumen and yolk percentage of quails (Table 2) was not significantly enhanced by vitamin A supplementation of feed. Watanabe (1999), Mendonça Júnior et al. (2002) and Mori et al. (2003) supplemented diets of laying hens with retinyl acetate and vitamin A, respectively, and did not notice differences on Haugh unit. On the other hand, Squires & Naber (1993) observed that the supplementation with vitamin A (9,000 UI/kg) in diets for laying hens resulted in the increase of Haugh unit when compared to control group.

The results for egg specific gravity, eggshell thickness, eggshell weight and eggshell percentage of quails (Table 2) did not present statistical differences. Possibly, the results of quails performance and internal and external quality of eggs were not altered by vitamin A supplementation in diets because vitamin requirements were already supplied by the control basal diet. Consequently, the excess of this vitamin was stored in the liver for later deposition on other tissues.

The retinol content in yolk presented a linear increase with the supplementation of vitamin A in diets of the birds (Table 2; Figure 1). The coefficient of determination, R^2=0.7985, indicates good adjustment of data to the line, meaning that 79.85% of data variability could be explained by the linear regression model.

Karadas et al. (2006) also reported the increase in concentration of vitamin A in yolk of eggs from quails by

Table 2 - Performance of quails, internal and external quality and enrichment of eggs from quails submitted to vitamin A supplementation in diet

Diet	Performance traits of birds				
	Daily feed intake (g)	Weight of eggs (g)	Egg production (%)	Food conversion/kg	Food conversion /dozen
Control	27.11	11.10	89.36	2.68	0.358
10,000 UI	27.28	10.94	90.52	2.68	0.351
20,000 UI	27.58	11.14	89.55	2.70	0.360
30,000 UI	27.61	11.10	88.44	2.76	0.367
Probability	0.08ns	0.62ns	0.55ns	0.31ns	0.33ns
F value	3.42	0.26	0.37	1.09	0.98
CV[1] (%)	1.89	2.17	3.99	4.73	5.64

	Internal quality traits of the eggs			
	Haugh unit	Yolk index	albumen %	yolk %
Control	84.89	0.444	61.61	30.54
10,000 UI	86.75	0.449	62.06	30.09
20,000 UI	86.03	0.443	61.77	30.37
30,000 UI	85.86	0.443	62.09	30.16
Probability	0.42ns	0.64ns	0.35ns	0.48ns
F values	0.66	0.22	0.90	0.52
CV[1] (%)	1.71	1.83	2.15	1.08

	External quality traits of the eggs			
	Specific gravity (g/cm^3)	Eggshell thickness (mm)	Eggshell weight (g)	Eggshell %
Control	1.069	0.240	0.882	7.85
10,000 UI	1.070	0.242	0.882	7.85
20,000 UI	1.070	0.243	0.897	7.86
30,000 UI	1.069	0.241	0.894	7.75
Probability	0.89ns	0.72ns	0.22ns	0.40ns
F values	0.02	0.13	1.56	0.74
CV[1] (%)	2.36	2.76	2.46	2.28

	Enrichment of eggs	
	Vitamin A in yolk (µg/g)	% of increase
Control	8.05	-
10,000 UI	25.47	216.34
20,000 UI	34.03	322.77
30,000 UI	51.22	536.27
Probability	0.001**	
F values	83.23	
CV[1] (%)	27.58	

** significant (P<0.001); ns – not significant (P>0.05); [1] coefficient of variation.

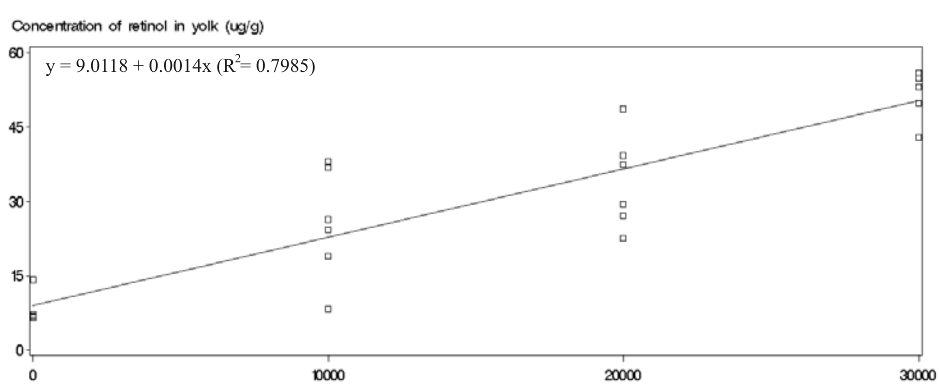

Figure 1 - Concentration of retinol in yolk (µg/g) of eggs from Japanese quails under supplementation with vitamin A (UI/kg).

supplementation with three sources of carotene (alfalfa, tomato powder, extract of calendula). Similarly, Watanabe (1999), adding crescent levels of vitamin A in diets of Hy-Line hens, verified progressive increase in levels of retinol in yolk of eggs, with percentages of increment which varied from 8.63 to 50.56%.

The cost of diet supplementation with 30,000 UI of vitamin A for quails was R$0.04 by kg of feed. By knowing that the recommendation of vitamin A for human beings is 2,615 UI/day, this would be equal to five quail enriched eggs. From this data, the cost of those five eggs to supply those requirements would be around R$0.27. But, a capsule containing the supplying recommendation of vitamin A/day costs, in average, R$0.14, thus there would be a higher cost of the same vitamin obtained from enriched eggs (27 cents). However, eggs can supply many other important nutrients, plus vitamin A, for human beings. Liberato & Sant'ana (2006) performed a study in Guatemala in order to achieve the recommended daily intake of vitamin A by people with high risk of deficiency of this vitamin and concluded that the per capita costs for enrichment was US$0.98; for the distribution of capsules was US$1.68 to US$1.86; and for programs of nutritional education that motivated the making of vegetable gardens was between US$3.10 to US$4.16. Retinol supplementation has no relation to the levels of cholesterol in eggs, therefore it is possible to produce enriched feed that promote beneficial effects to health without unfavorable side effects.

The results from vitamin D supplementation did not display statistical difference in eggs weight, egg production and food conversion (by kg and by dozen of eggs) of quails during experimental period (Table 3). However, feed intake increased significantly with the supplementation with vitamin D, presenting quadratic effect. Intake increased to the level 1,034 UI of vitamin D/kg of feed (Figure 2). Value of coefficient of determination (R^2=0.4259) evidenced that the model was not able to explain great part of the variation in feed intake.

Similar results were reported by Frost & Roland (1990) using increasing levels of vitamin D_3 (0; 500; 1,000 and 1,500 UCI/kg). Those authors verified increase in eggs production and feed intake when the level of vitamin D_3 increased in the feed. However, the egg weight was not influenced by the levels of vitamin D_3 supplied to birds.

Table 3 - Performance traits of quails, internal and external quality and enrichment of eggs from quails receiving supplementation of vitamin D in diet

Diet	Performance of birds				
	Daily feed intake (g)	Eggs weight (g)	Egg production (%)	Food conversion/kg	Food conversion/dozen
Control	26.61	11.43	81.55	2.87	0.393
500 UI	27.19	11.55	84.98	2.78	0.385
1,000 UI	27.18	11.43	84.90	2.82	0.388
1,500 UI	27.18	11.56	85.27	2.80	0.385
Probability	0.02*	0.61ns	0.36ns	0.71ns	0.71ns
F values	7.79	0.27	0.88	0.14	0.15
CV[1] (%)	1.09	2.34	7.67	7.87	8.48

	Internal quality of the eggs			
	Haugh unit	Yolk index	albumen %	yolk %
Control	91.33	0.499	61.41	30.78
500 UI	90.78	0.496	61.49	30.89
1,000 UI	91.31	0.493	61.48	30.93
1,500 UI	90.67	0.490	61.51	31.15
Probability	0.57ns	0.13ns	0.87ns	0.53ns
F values	0.33	2.45	0.03	0.41
CV[1] (%)	1.51	2.20	1.62	3.21

	External quality of eggs			
	Specific gravity (g/cm^3)	Eggshell thickness (mm)	Eggshell weight (g)	Eggshell %
Control	1.065	0.249	0.910	7.82
500 UI	1.065	0.247	0.897	7.63
1,000 UI	1.066	0.245	0.901	7.59
1,500 UI	1.064	0.243	0.885	7.59
Probability	0.57ns	0.15ns	0.22ns	0.07ns
F values	0.33	2.21	1.60	3.58
CV[1] (%)	1.06	2.87	3.41	2.63

	Enrichment of eggs	
	Vitamin D in yolk (µg/g)	% of increase
Control	537.03	-
500 UI	557.20	3.75
1,000 UI	560.20	4.32
1,500 UI	609.10	13.43
Probability	0.001**	
F values	42.82	
CV[1] (%)	2.63	

* significant (P<0.05); ** significant (P<0.001); ns – not significant (P>0.05); [1] coefficient of variation.

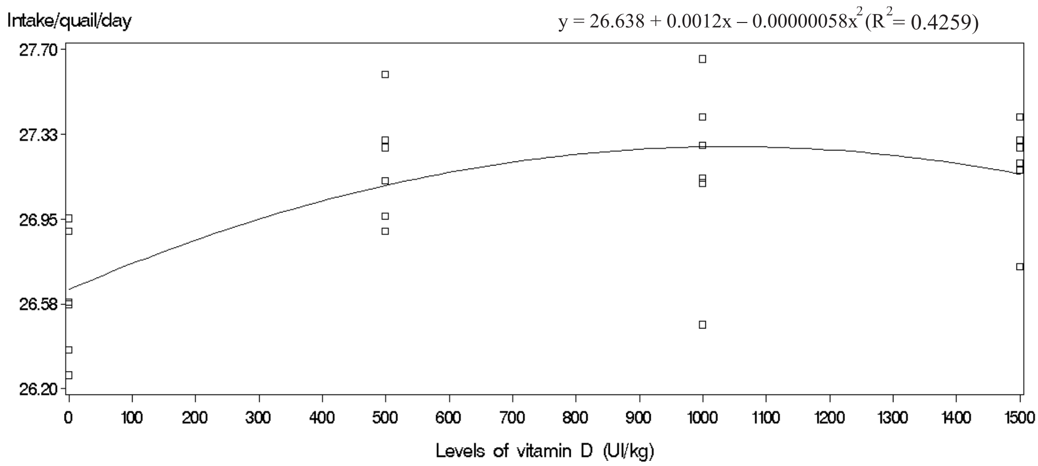

Figure 2 - Feed intake (quail/day) of Japanese quails under vitamin D supplementation (UI/kg).

Disaccording with these results, Salvador et al. (2009) verified that feed intake, eggs production, egg weight and egg mass were not affected by supplementation of diets for laying hens with two sources of vitamin D and three levels of vitamin C. Those reports coincide with the results found by Torres et al. (2009), who did not observe significant differences in the production of eggs from female broiler breeders supplemented with 25-hidrocholecalciferol associated with vitamin D_3.

Results presented in this experiment for food conversion differ from the ones found by Salvador et al. (2009), who found better values for food conversion when laying hens were fed with the metabolite 25(OH)D_3 without the inclusion of vitamin C.

The absence of effects from vitamin D levels used in this experiment in some performance parameters was also reported by Mattila et al. (2004) in a work with different levels and sources of vitamin D evaluated in laying hens at 20 weeks of age.

Vitamin D supplementation in feed was not determinant for a significant enhance of internal quality of eggs for the traits Haugh unit, yolk index, albumen and yolk percentage (Table 3). These results confirmed the reports by Hernández et al. (2001) who found that the Haugh unit was not influenced by the supplementation of 25(OH)D_3 with two levels of calcium (high and low) in eggs from laying hens of 1st and 2nd laying cycle.

When evaluating the effect of two sources of vitamin D (cholecalciferol and 25-hidroxicholecalciferol) and three levels of vitamin C (0, 100 and 200 ppm), Salvador et al. (2009) observed that the higher percentage of albumen was observed when diets were supplemented with 200 ppm of vitamin C in association to cholecalciferol. However, for the percentage of yolk, the higher value was verified when supplemented with 200 ppm of vitamin C in association to metabolite 25 (OH)D_3.

There were no significant difference between the results from treatments (Table 3) for external quality of egg (specific gravity, eggshell thickness, eggshell weight and eggshell percentage) agreeing with the results from Rodrigues et al. (2005a), who conducted an experiment with three levels of vitamin D (1,200; 2,400 and 3,600 UI/kg) in pre-lay feed and two levels of vitamin D (1,200 and 2,400 UI/kg) in a diet for laying phase, and verified that the eggshell quality, eggshell percentage, eggshell thickness and eggs specific gravity from laying hens, regardless of supplying phase, were not altered by different levels of vitamin D in diet.

Different results from this experiment were reported by Rodrigues et al. (2005b), who supplemented diet of laying hens with three levels of calcium (1.3; 1.8 and 2.3%) and two levels of vitamin D (1,200 and 2,400 UI/kg), and verified that the best specific gravity was obtained with the higher level of vitamin D in the diet regardless to the calcium levels used.

Results from this research related to thickness and percentage of eggshell are in agreement with the ones found by Faria et al. (2000), who supplemented diet of laying hens with different levels of energy, vitamin D_3 and sodium:chloride ratio and observed that eggshell percentage and eggshell thickness did not present significant differences. However, Faria et al. (1999), using three levels of vitamin D in diet for laying hens (2,500; 3,000 and 3,500 UI/kg), found better eggshell thickness when birds were submitted to diets containing 2,500 and 3,000 UI/kg.

The increase of cholecalciferol content in egg yolk according to the level of vitamin D supplementation in diet (Table 3) presented linear regression: $y = 534.82 + 0.0427x$; $R^2 = 0.7536$ (Figure 3), evidencing that the level of cholecalciferol increased as the supplementation of vitamin

D in diet for quails increased. Coefficient of determination ($R^2 = 0.7536$) indicated good adjustment of data to the line, meaning that 75.36% of the data variability could be explained by the linear regression model.

Results from this experiment are in agreement with the ones obtained by Mattila et al. (1999), who studied the effect of supplementation in diet for laying hens with three levels of vitamin D_3 (1,064; 2,496 and 8,640 UI/kg) on the concentration of cholecalciferol and 25-hidroxicholecalciferol in yolk of eggs from laying hens and observed positive correlations between cholecalciferol in diet and cholecalciferol (r = 0.995) and 25-hidroxicholecalciferol (r=0.941) contained in yolk. Similarly, Mattila et al. (2004), supplementing diet of laying hens with vitamin D (D_2 and D_3), observed 33.20% increase of vitamin D_3 concentration in egg yolk for the vitamin level of 15,000 UI/kg in diet.

Diet supplementation of quails with 1,500 UI of vitamin D cost R$0.0096 by kilogram of feed. The recommendation of vitamin D for human being is 200 UI/day, which would be equal to four enriched eggs of quails. Thus, the cost of these four eggs to attend the requirements would be around 22 cents of Real. A capsule containing the recommended intake of vitamin D/day costs, in average, R$0.20, therefore the enrichment of eggs would be economically viable. Also, an egg can supply other vitamins, minerals and essential proteins plus vitamin D.

The levels of vitamin E added to the diet did not result in significant effects on performance traits of birds (Table 4). These founds are in agreement with the results found by Barreto et al. (1999), who did not observe significant differences on productive performance of laying hens submitted to increasing levels of vitamin E in diet.

Similarly, Meluzzi et al. (2000) did not notice significant differences on productive performance of laying hens supplemented with 50, 100 and 200 mg of DL-α-tocopheryl/kg of diet. However, those results are in disagreement with the ones found by Scheideler & Froning (1994), who verified enhance of 2% in eggs production of laying hens supplemented with 50 mg of vitamin E/kg of feed, and Mori et al. (2003) who observed the worst results for eggs production when laying hens were supplemented with 600 mg of vitamin E/kg of feed. Results obtained in this study differ from the ones found by Bolukbasi et al. (2007) for egg production when supplementing diets of laying hens with 0, 45, 65 and 85 UI of vitamin E/kg of feed.

According to Mori et al. (2003), the supplementation with 200, 400 and 600 mg of vitamin E/kg of feed did not influence feed intake and egg weight, but it worsen food conversion (kg and dozen of eggs).

Vitamin E supplementation in feed did not alter significantly the internal quality of eggs, evaluated by Haugh unit, yolk index and albumen and yolk percentages (Table 4). These results are in agreement with the ones found by Barreto et al. (1999), who analyzed the effect of supplementation of 25 and 250 mg of vitamin E/kg of diet for female broiler breeders and did not observe significant differences for albumen and yolk percentages during the studied period. Different results were found by Yardibi & Turkay (2008), who supplemented diets of laying hens with three levels of vitamin E (30, 80 and 105 mg/kg) submitting birds to heat stress (35°C) and observed significant decrease of Haugh unit, weight and specific gravity of eggs during the experimental period.

External quality of eggs (specific gravity, eggshell thickness, eggshell weight and eggshell %) did not present significant difference with the levels of supplementation, which is in accordance with the observations of Almeida (2001) and Mori et al. (2003), who supplemented 200, 400

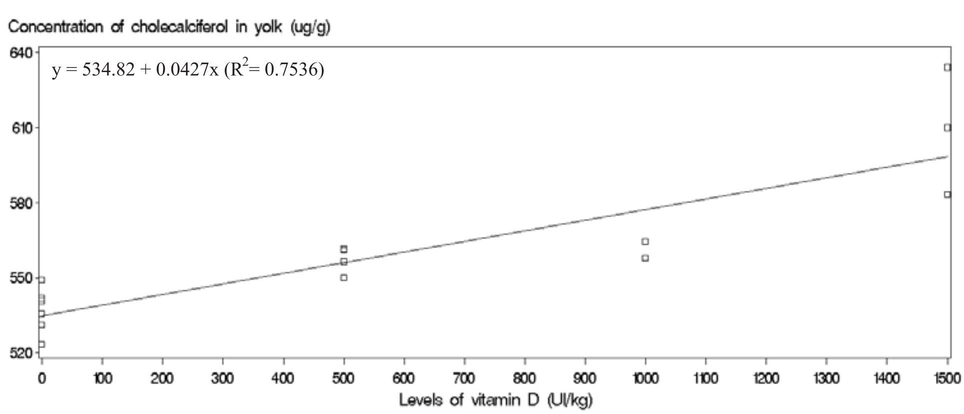

Figure 3 - Concentration of cholecalciferol in yolk (µg/g) of eggs from Japanese quails under supplementation of vitamin D (UI/kg) in the diet.

Table 4 - Performance traits of quails, internal and external quality and egg enrichment of quails under supplementation of vitamin E in the diet

Diet	Performance traits				
	Daily feed intake (g)	Egg weight (g)	Egg production (%)	Food conversion/kg	Food conversion/dozen
Control	27.36	11.05	85.85	2.98	0.394
200 UI	27.83	11.13	89.20	2.94	0.393
400 UI	27.05	11.13	87.85	2.81	0.375
600 UI	26.80	11.17	87.41	2.78	0.372
Probability	0.23ns	0.33ns	0.79ns	0.16ns	0.21ns
F values	1.49	1.00	0.07	2.07	1.65
CV[1] (%)	4.07	1.76	7.64	9.56	9.51

	Internal quality of the eggs			
	Haugh unit	Yolk index	albumen %	yolk %
Control	85.49	0.453	62.21	29.78
200 UI	87.29	0.450	61.61	30.32
400 UI	86.98	0.460	61.95	30.09
600 UI	86.74	0.460	61.55	30.53
Probability	0.40ns	0.18ns	0.35ns	0.25ns
F values	0.75	1.91	0.92	1.42
CV[1] (%)	1.87	3.08	1.50	3.09

	External quality of eggs			
	Specific gravity (g/cm^3)	Eggshell thickness (mm)	Eggshell weight (g)	Eggshell %
Control	1.070	0.247	0.888	7.97
200 UI	1.071	0.244	0.921	8.05
400 UI	1.071	0.243	0.891	7.97
600 UI	1.070	0.243	0.888	7.92
Probability	0.98ns	0.42ns	0.57ns	0.40ns
F values	0.01	0.66	0.33	0.74
CV[1] (%)	2.12	2.95	3.32	1.87

	Enrichment of eggs	
	Vitamin E in yolk (mg/g)	% of increase
Control	0.19	-
200 UI	0.58	203.14
400 UI	0.79	313.08
600 UI	1.10	479.05
Probability	0.001**	
F values	273.52	
CV[1] (%)	14.68	

** significant (P<0.001); ns – not significant (P>0.05); [1] coefficient of variation.

and 600 UI of vitamin E/kg of diet for laying hens and did not notice significant differences on eggshell weight. Similarly, Qi & Sim (1998) and Galobart et al. (2001), using diets containing between 0 and 800 mg of vitamin E/kg of feed, did not verify significant differences on eggshell weight. As counterpart, Pita et al. (2004) supplemented diets of laying hens with 0, 100 and 200 UI of vitamin E and observed eggshell weight significantly higher than the eggs from the group that did not receive supplementation.

The results found for performance of quails and internal and external quality of eggs from quails submitted to supplementation of diet with vitamin E are probably related to vitamin supplementation used in basal diet, which supplied the requirements of vitamin E of birds and, then, did not cause alterations in this traits.

Tocopherol content in yolk increased according to the supplementation of vitamin E in diet of birds (Table 4). The level of tocopherol increased linearly ($y = 0.223 + 0.0015x$; $R^2 = 0.9256$; Figure 4) as the supplementation of vitamin E in diet of quails increased. Coefficient of determination ($R^2 = 0.9256$) indicated excellent adjustment of data to the line, meaning that 92.56% of data variability could be explained by the linear regression model.

These results are in agreement with the ones found by Meluzzi et al. (2000), who supplemented diets of laying hens with two kinds of oils and four levels of vitamin E (0, 50, 100 and 200 ppm), and observed linear increase of acetate DL-α-tocopheryl (P<0.01) in yolk of eggs. In the same way, Shariar et al. (2008) observed linear increase of the tocopherol incorporation in yolk of eggs from heavy female broiler breeders submitted to supplementation of vitamin E and fat in diet.

These results can be explained because the liver plays a predominant role on the metabolism of lipids and

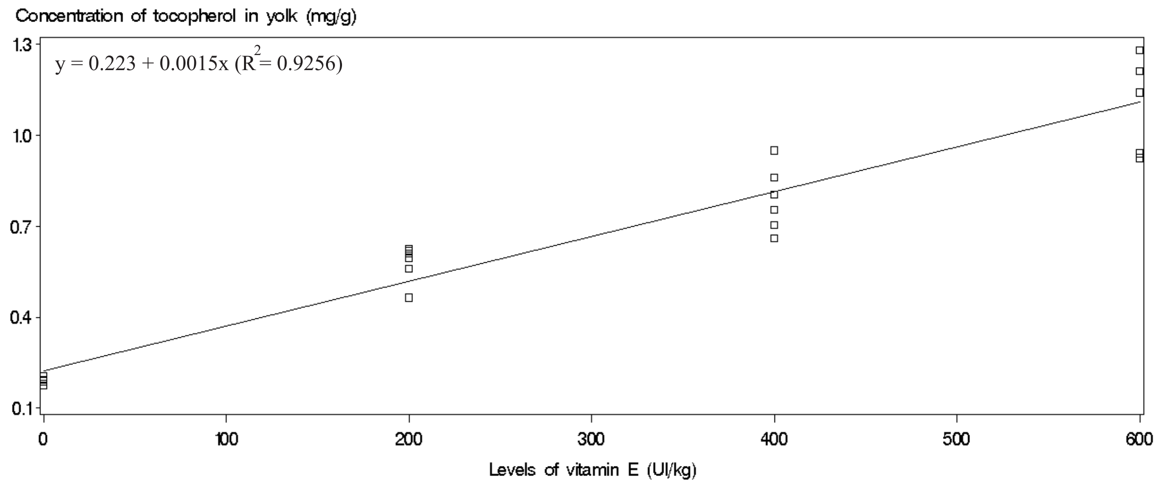

Figure 4 - Concentration of tocopherol in yolk (mg/g) of Japanese quails under supplementation with vitamin E (UI/kg).

lipossoluble vitamins in birds due to their rudimentary lymphatic system (Kang et al., 1998). The presence of a specific protein into the hepatocytes responsible for the retention of α-tocopherol in the liver was described in humans (Traber & Sies, 1996) and the results observed in the present research suggest that a preferential hepatic retention exists for α-tocopherol also in birds. Tocopherols in liver would be transported as a component of blood VLDL (very low density protein) directly to the yolk of eggs (Cherian et al., 1996b). In birds, VLDL does not pass by a considerable lipolysis process in its route from the liver to the oocytes (Nimpf & Schneider, 1991) thus any significant losses of tocopherols occur in this transport, which justifies the high efficiency of feed tocopherols transfer to the yolk of eggs. According to Surai et al. (1998b), each egg has an amount of vitamin E that is as twice as higher the amount of this vitamin stored in the liver. Therefore, laying hens are the most efficient species among other domestic birds in tocopherol transfer from diet to oocyte because the liver is not a vitamin E reservoir for them (Surai et al., 1998a).

Supplementation in diet of quails with 600 UI of vitamin E presented cost of R$0.08 by kg of feed. The recommendation of vitamin E for human beings is 15 UI/day, what would be equal to five enriched eggs of quails. Therefore, the cost of those five eggs to attend the requirements would be around R$0.28. However, a capsule containing the recommended daily intake of vitamin E costs, in average, R$0.17, still the enrichment of eggs is economically viable, once the egg can supply other essential nutrients for human beings plus vitamin E.

Conclusions

Supplementation of vitamins A, D and E in diet of Japanese quails do not worsen the productive performance neither the internal and external quality of the eggs, except the supplementation of vitamin D which increases intake. Supplementation with theses vitamins is efficient for cholecalciferol, retinol and tocopherol incorporation in yolk, evidencing that the nutritional value of egg, related to vitamins A, D and E can be increased by the supplementation in diet of quails.

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References

ALMEIDA, C.R.M. **Influência da vitamina E alimentar no enriquecimento de ovos de galinhas**. 2001. 60f. Dissertação (Mestrado em Clínica Veterinária) – Universidade de São Paulo. Faculdade de Medicina Veterinária e Zootecnia. Departamento de Clínica Médica, São Paulo, 2001.

BÁRDOZ, L.; SÓTER, G.Y.; KARCHESZ, K. Effect of retinyl acetate, ascorbic acic and tocopherol supplementation of the feed on egg vitamin A content in Japanese quail. **Acta Veterinaria Hungarica**, v.44, n.2, p.213-219, 1996.

BARRETO, S.L.T.; FERREIRA, W.M.; GONÇALVES, T.M. Níveis de proteína e de vitamina E para matrizes de frango de corte.

1. Efeito sobre o desempenho das matrizes, composição do ovo e desempenho da progênie. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v.51, n.2, p.183-192, 1999.

BATISTA, E.S.; COSTA, A.G.V.; PINHEIRO-SANT'ANA, H.M. Adição da vitamina E aos alimentos: implicações para os alimentos e para a saúde humana. **Revista de Nutrição**, v.20, p. 525-535, 2007.

BOLUKBASI, S.C.; ERHAN, M.K.; KELES, M.S. et al. Effect of dietary vitamin E on the performance, plasma and egg yolk vitamin E levels and lipid oxidation of egg in heat stressed layers. **Journal of Applied Biological Sciences**, v.1, n.3, p.19-13, 2007.

CARD, L.E.; NESHEIM, M.C. **Poultry production**. Philadelphia: Lea & Febiger, 1966. 399p.

CHERIAN, G.; WOLFE, F.H. SIM, J.S. Feeding dietary oils with tocopherols: Effects on internal qualities of eggs during storage. **Journal of Food Science**, v.61, n.1, p.15-17, 1996a.

CHERIAN, G.; WOLFE, F.H. SIM, J.S. Dietary oils with added tocopherols: effects on egg or tissue tocopherols, fatty acids, and oxidative stability. **Poultry Science**, v.75, p.423-431, 1996b.

FARIA, D.E.; JUNQUEIRA, O.M.; SOUZA, P.A. et al. Influência de diferentes níveis de vitaminas D e C e idade das galinhas poedeiras sobre o desempenho e qualidade dos ovos. 1 – Verão. **Revista Brasileira de Ciência Avícola**, v.1, n.3, p.193-201, 1999.

FARIA, D.E.; JUNQUEIRA, O.M.; SAKOMURA, N.K. et al. Influência de diferentes níveis de energia, vitamina D_3 e relação sódio:cloro sobre o desempenho e a qualidade de casca de ovos de poedeiras comerciais. **Revista Brasileira de Zootecnia**, v.29, n.2, p.467-475, 2000.

FROST, T.J.; ROLAND Sr., D.A. Influence of vitamin D_3, 1α-hydroxyvitamin D_3 and 1,25-dihydroxyvitamin D_3 on egg shell quality, tibia strength, and various production parameters in commercial laying hens. **Poultry Science**, v.69, p.2008-2016, 1990.

FU, Z.; KATO, H.; SUGAHARA, K. et al. Retinoic acid accelerates the development of reproductive organs and egg production in japanese quail (*Coturnix coturnix japonica*). **Biology of Reproduction**, v.63, n.6, p.1795-1800, 2000.

GALOBART, J.; BARROETA, A.C.; BAUCELLS, M.D. et al. Lipid oxidation in fresh and spray-dried eggs enriched with n-3 and n-6 polynsaturated fatty acid during storage affected by dietary vitamin E and cathaxanthin supplementation. **Poultry Science**, v.80, p.327-337, 2001.

HERNÁNDEZ, M.G.; LÓPEZ, R.M.; GONZÁLEZ, E.A. et al. Mejoramiento de la calidad del cascarón con 25 hidroxicolecalciferol [25-(OH)D_3] en dietas de gallinas de primero y segundo ciclos. **Veterinária México**, v.32, p.167-174, 2001.

INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA – IBGE. **Sistema IBGE de recuperação automática**. Disponível em: <http://www.ibge.gov.br/home/presidencia/noticias/noticia_visualiza.php?id_noticia=1272&id_pagina=1> Acesso em: 7/12/2009.

KANG, K.R.; CHERIAN, G.; SIM, J.S. Tocopherols, retinol and carotenes in chicken egg and tissues as influenced by dietary palm oil. Journal of Food Science, v.63, p.592-596, 1998.

KARADAS, F.; SURAI, P.F.; SPARKS, N.H.C. et al. Effects of maternal dietary supplementation with three sources of carotenoids on the retinyl esters of egg yolk and developing quail. **Comparative Biochemistry and Physiology – A**, v.140, p.430-435, 2006.

LIBERATO, S.C.; SANT'ANA, H.M. Fortification of industrialized foods with vitamins. **Revista de Nutrição**, v.19, n.2, p.215-231, 2006.

LIN, H.; WANG, L.F.; SONG, J.L. et al. Effect of dietary supplemental levels of vitamin A on the egg production and immune responses of heat-stressed laying hens. **Poultry Science**, v.81, p.458-465, 2002.

MATTILA, P.; LEHIKOINEN, K.; KIISKINEN, T. et al. Cholecalciferol and 25-hydroxycholecalciferol content of chicken egg yolk as affected by the cholecalciferol content of feed. **Journal of Agricultural and Food Chemistry**, v.47, p.4089-4092, 1999.

MATTILA, P.; ROKKA, T.; KONKO, K. et al. Effect of cholecalciferol-enriched hen feed on egg quality. **Journal of Agricultural and Food Chemistry**, v.51, p.283-287, 2003.

MATTILA, P.; VALAJA, J.; ROSSOW, L.T. et al. Effect of vitamin D_2 and D_3 enriched deits on egg vitamin D content, production, and bird condition during an entire production period. **Poultry Science**, v.83, p.433-440, 2004.

MELUZZI, A.; SIRRI, F.; MANFREDA, G. et al. Effects of dietary vitamin E on the quality of table eggs enriched with n-3 long-chain fatty acids. **Poultry Science**, v.79, p.539-545, 2000.

MENDONÇA JÚNIOR, C.X.; ALMEIDA, C.R.M.; MORI, A.V. et al. Effect of dietary vitamin A on egg yolk retinol and tocopherol levels. **Journal of Applied Poultry Research**, v.11, p.373-378, 2002.

MILLWARD, D.J. Macronutrient intakes as determinants of dietary protein and amino acid adequacy. **The Journal of Nutrition**, v.134, p.1588S-1596S, 2004.

MORI, A.V.; MENDONÇA JÚNIOR., C.X.; ALMEIDA, C.R.M. et al. Supplementing hen diets with Vitamins A and E affects egg yolk retinol and *á*-tocopherol levels. **Journal of Applied Poultry Research**, v.12, p.106-114, 2003.

MURAKAMI, A.E.; MORAES, V.M.B.; ARIKI, J. et al. Níveis de proteína e energia em rações para codornas (*Coturnix coturnix japonica*) em postura. **Revista Brasileira de Zootecnia**, v.22, n.4, p.541-551, 1993.

MURAKAMI, A.E.; GARCIA, E.R.M. Pontos críticos na criação de codornas. In: CURSO DE ATUALIZAÇÃO EM AVICULTURA PARA POSTURA, 4., 2007, Jaboticabal, **Anais...** Jaboticabal: FCAV, 2007. p.41-53.

NATIONAL RESEARCH COUNCIL - NRC. **Nutrient requirements of poultry**. 9.rev.ed. Washington, D.C.: National Academy Press, 1994. 155p.

NIMPF, J.; SCHNEIDER, W.J. Receptor-mediated lipoprotein transport in laying hens. **The Journal of Nutrition**, v.121, n.9, p.1475-1485, 1991.

PITA, M.C.G.; PIBER NETO, E.; NAKAOKA, L.M. et al. Efeito da adição de ácidos graxos insaturados e de vitamina E à dieta de galinhas e seu reflexo na composição lipídica e incorporação de α-tocoferol na gema do ovo. **Brasilian Journal of Veterinary Research and Animal Science**, v.41, n.1, p.25-31, 2004.

QI, G.H.; SIM, J.S. Natural tocopherol enrichment its effects in n-3 fatty acid modified chicken eggs. **Journal of Agriculture Food Chemistry**, v.46, p.1920-1926, 1998.

RAMALHO, H.M.M.; DIAS DA SILVA, K.H.; ALVES DOS SANTOS, V.V. et al. Effect of retinyl palmitate supplementation on egg yolk retinol and cholesterol concentrations in quail. **British Poultry Science**, v.49, n.4, p.475-481, 2008.

REID, B.L.; HEYWANG, B.W.; KURNICK, A.A. et al. Effect of vitamin A and ambient temperature on reproductive performance of white leghorn pullets. **British Journal of Nutrition**, v.14, p.211-216, 1965.

RODRIGUES, E.A.; JUNQUEIRA, O,M.; CANCHERINI, L.C. et al. Desempenho e qualidade da casca para poedeiras recebendo vitamina D nas rações pré-postura e postura. **Acta Scientiarum Animal Sciences**, v.27, p.55-59, 2005a.

RODRIGUES, E.A.; JUNQUEIRA, O.M.; ANDREOTTI, M.O. et al. Níveis de cálcio e vitamina D nas rações de pré-postura sobre o desempenho e qualidade de casca de ovos de poedeiras comerciais. **Acta Scientiarum Animal Sciences**, v.27, p.61-66, 2005b.

ROSTAGNO, H.S.; ALBINO, L.F.T.; DONZELE, J.L. et al. **Tabelas brasileiras para aves e suínos. Composição de alimentos e exigências nutricionais**. 2.ed. Viçosa, MG: UFV, 2005. 186p.

SALVADOR, D.; FARIA, D.E.; MAZALLI, M.R. et al. Vitaminas D e C para poedeiras na fase inicial de produção de ovos. **Revista Brasileira de Zootecnia**, v.38, p.887-892, 2009.

SCHEIDELER, S.E.; FRONING, G. Dietary flaxseed improves egg production and incorporation of omega 3 fatty acids in eggs. **Poultry Science**, v.73, p.50-56, 1994.

SHAHRIAR, H.A.; ADL, K.N.; NEZHAD, Y.E. et al. Effect of dietary fat type and different levels of vitamin E on broiler breeder performance and vitamin E levels of egg. **Asian Journal of Animal and Veterinary Advances**, v.3, n.3, p.147-154, 2008.

SQUIRES, M.W.; NABER, E.C. Vitamin profiles of eggs as indicators of nutritional status in the laying hen: vitamin A study. **Poultry Science**, v.72, p.154-164, 1993.

SURAI, P.F.; IONOV, I.A.; KUCHMISTOVA, E.F. et al. The relationship between the levels of α-tocopherol and carotenoids in the maternal feed, yolk and neonatal tissues: comparison between the chicken, turkey, duck and goose. **Journal of the Science of Food and Agriculture**, v.76, n.4, p.593-598, 1998a.

SURAI, P.F.; IONOV, I.A.; KUKLENKO, T.V. et al. Effect of supplementing the hen´s diet with vitamin A on the accumulation of vitamin A and E, ascorbic acid and carotenoids in the egg yolk and in the embryonic liver. **British Poultry Science**, v.39, n.2, p.257-263, 1998b.

TORRES, C.A.; VIEIRA, S.L.; REIS, R.N. et al. Productive performance of broiler breeder hens fed 25-hydroxycholecalciferol. **Revista Brasileira de Zootecnia**, v.38, p.1286-1290, 2009.

TRABER, M.G.; SIES, H. Vitamin E in humans: demand and delivery. **Annual Reviews of Nutrition**, v.53, n.1, p.335-345, 1996.

WATANABE, C. **Efeito da adição de vitamina A à ração sobre as concentrações de retinol e tocoferol no plasma e na gema de ovo de galinhas**. 1999. 78f. Dissertação (Mestrado em Clínica Veterinária) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo.

YARDIBI, H.; TURKAY, G. The effects of vitamin E on the antioxidant system, egg production, and egg quality in heat stressed laying hens. **Turkish Journal of Veterinary and Animal Science**, v.32, n.5, p.319-325, 2008.