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Original Article

Fat-Soluble Vitamin Supplementation Levels in Diets for Laying Hens from 28 to 44 Weeks of Age

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ABSTRACT

This study aimed to examine the impact of levels of dietary supplementation with fat-soluble vitamins on the production performance and egg quality of laying hens. Three hundred Hy-Line White W-36 laying hens were evaluated from 28 to 44 weeks of age. The birds were allotted to one of six treatments in a randomized block design with 10 replicates with five birds each. Performance and egg quality parameters were evaluated in four 28-day periods. A corn and soybean meal-based basal diet was formulated so as to meet the nutritional requirements of the animals, with the exception of fat-soluble vitamins. The treatments consisted of dietary supplementation with 0%, 33.3%, 66.7%, 100.0%, 133.3% or 166.7% of fat-soluble vitamins (100% supplementation consisted of 7500 IU, 2000 IU, 10 IU and 1.8 mg of vitamins A, D_3, E and K per kilogram of diet, respectively). Eggshell weight, shell thickness, shell strength, feed intake, egg weight, feed conversion per egg mass and feed conversion per dozen eggs showed a quadratic response ($p \leq 0.05$) to the treatments, whereas egg mass responded linearly. Optimal results were obtained at an average fat-soluble vitamin supplementation level of 109%, which corresponds to 8175 IU of vitamin A, 2180 IU of vitamin D_3, 10.9 IU of vitamin E and 1.96 mg of vitamin K per kilogram of diet.

INTRODUCTION

Vitamins are nutrients required in small amounts (milligrams or micrograms) which may or may not be synthesized by the animal´s organism. They are classified according to their physiological functions in the body and how they contribute to the maintenance of health. Vitamins are essential for the different development phases of a bird, and their absence in the diet, or low absorption, may induce signs of metabolic deficiency (Barroeta *et al.*, 2012).

Fat-soluble vitamins (A, D, E and K) have a primary role in the bird´s metabolism. These vitamins are digested and absorbed through the same pathway as fats because they are associated with food lipids and are stored in tissues like the liver and adipose tissue (Barroeta *et al.*, 2012).

According to Rostagno *et al.* (2011), the recommended fat-soluble vitamin supplementation levels to ensure satisfactory performance are 7500 IU of vitamin A, 2000 IU of vitamin D_3, 10 IU of vitamin E and 1.8 mg of vitamin K per kilogram of diet. However, the National Research Council (NRC, 1994) recommends supplementing 2500 IU, 250 IU, 4 IU and 0.4 mg of vitamins A, D_3, E and K_3 per kilogram of diet. Inclusion levels vary considerably between recommendation tables, line manuals and companies' recommendations. Published requirements – especially by the NRC – indicate minimum values to prevent vitamin deficiency without considering maximum animal performance. However, in laying-hen farming systems, vitamins may be supplemented at levels higher than recommended in an attempt to meet the requirements of those birds, which are under constant stress conditions and may thus need a greater vitamin uptake.

Therefore, questions still remain regarding the optimal levels to be adopted to obtain better results in intensive layer-farming systems; i.e., to achieve the maximum production potential allowed by the genetics of current lines. On this basis, the present study examined levels of supplementation with fat-soluble vitamins for laying hens from 28 to 44 weeks of age.

MATERIALS AND METHODS

All experimental procedures were approved by the Ethics Committee on the Use of Production Animals (CEUAP) at the Federal University of Viçosa (UFV) (approval no. 057/2015).

Three hundred Hy-Line White W-36 laying hens were used in the experiment. The birds were acquired at 18 weeks of age and housed in cages where they received a diet formulated as recommended by the Brazilian Tables for Poultry and Swine (Rostagno *et al.*, 2011) until the start of the experimental period, at 28 weeks of age.

The cages were located in a brick shed with a ceiling height of 1.8 m and clay-tile roofing. The shed was equipped with conventional laying cages (25×45×40 cm) arranged in two overlapping rows, with a 1.5-m-wide central corridor between the rows. Feed and water were available *ad libitum* and the environment was illuminated for 17 h during the entire experimental period, in accordance with the management recommendations for the line.

Air temperature was measured daily using bulb thermometers which had been installed at different points in the shed, at the height of the birds. The minimum and maximum temperatures recorded during the experimental period were 19.8ºC±1.0 and 30.8ºC±2.1, respectively.

Birds were allotted to six treatments in a randomized-block design (white-egg layers: 1.28±0.03 kg; white-egg layers: 1.44±0.04 kg) with 10 replicates and five chickens per experimental unit, in the period of 28 to 44 weeks of age.

The experimental diets had the same nutritional value. A corn and soybean meal-based basal diet was formulated to meet the nutritional requirements of the animals in accordance with the Brazilian Tables for Poultry and Swine (Rostagno *et al.*, 2011) (Table 1).

Table 1 – Basal-diet composition (fresh-matter basis).

Ingredient	Dietary inclusion (g/kg)
Corn	549.8
Soybean meal, 45%	246.2
Corn gluten meal, 60%	35.0
Soybean oil	36.4
Salt	5.7
Calcitic limestone	102.3
Dicalcium phosphate	12.6
L-lysine HCL, 79%	1.23
DL-methionine, 99%	2.76
L-threonine, 98%	0.57
L-valine, 98.5%	0.65
Vitamin supplement (fixed)[1]	1.67
Mineral supplement[2]	1.10
Vitamin supplement + starch (variable)[3]	3.56
Antioxidant[4]	0.10
Calculated composition	
Metabolizable energy, kcal/kg	2.900
Crude protein (g/kg)	179.9
Calcium (g/kg)	43.2
Available phosphorus (g/kg)	3.23
Digestible lysine (g/kg)	8.66
Digestible threonine (g/kg)	1.99
Digestible met. + cys. (g/kg)	7.88
Digestible tryptophan (g/kg)	1.99
Digestible leucine (g/kg)	16.43
Digestible valine (g/kg)	8.23
Digestible gly. + ser. (g/kg)	14.45

[1]Content per kg of diet: vit. B1, 1.5 mg; vit. B2, 4 mg; vit. B6, 1.7 mg; vit. B12, 0.013 mg; pantothenic acid, 10 mg; nicotinic acid, 25 mg; folic acid, 0.5 mg; biotin, 0.05 mg; choline, 0.43 mg.

[2]Content per kg of diet: copper, 10 mg; iron, 50 mg; iodine, 1 mg; manganese, 70 mg; selenium, 0.3 mg; zinc, 65 mg.

[3]Content per kg of diet: vit. A, 7500 IU; vit. D3, 2000 IU; vit. E, 10 IU; vit. K3, 1.8 mg

[4]Butylated hydroxytoluene (BHT).

The treatments consisted of dietary supplementation with 0%, 33.3%, 66.7%, 100.0%, 133.3% or 166.7% of fat-soluble vitamins. The treatment considered 100% inclusion was based on the recommendations of Rostagno *et al.* (2011), containing 7500 IU vitamin A, 2000 IU vitamin D_3, 10 IU vitamin E, and 1.8 IU vitamin K_3 per kilogram of diet.

The following performance and egg-quality variables were evaluated during four 28-day periods: weight gain (WG, g/bird/day), feed intake (FI, g/bird/day), egg production (EP, g/kg), egg weight (EW, g), egg mass (EM, g), feed conversion per egg mass (CEM, g/g), and feed conversion per dozen eggs (CDZ, kg/dz).

Haugh unit was measured using an EggTester® apparatus. The device relates albumen height to egg weight using the following formula:

$HU = 100*\log(h - 1.7w^{0.37} + 7.6)$,

where *h* = albumen height, in millimeters; and *w* = egg weight, in grams.

Specific gravity (GRAV, g/cm³): samples of healthy eggs were immersed in NaCl solutions of densities ranging from 1.050 to 1.100 g/cm³ with a gradient of 0.005 between the densities. A densimeter was used to determine the values, and this variable was expressed in g/cm³ (Freitas *et al.*, 2004).

Egg components (g/kg): the eggs were cracked and the yolk (YLK) and albumen (ALB) were separated. The YLK of each experimental unit were weighed together to obtain their average weight. The eggshells (SHL) were washed, dried in a forced-air oven at 60 °C and subsequently weighed in the same way to calculate average shell weight. Average albumen weight was calculated by difference (Ahn *et al.*, 1997).

Eggshell strength (SHLS, Newtons): strength measured using an EggTester® apparatus upon compressing the two ends of the egg until the shell cracked.

Eggshell thickness (SHLT, mm): measured using a caliper.

All data were subjected to ANOVA using the PROC GLM procedure of SAS statistical package (SAS Institute, 2010). Orthogonal polynomial contrasts were used in the analysis of variance to evaluate the effect of treatments and PROC REG was used to estimate the regression equations. The linear and quadratic equations were adjusted to interpret the data when $p \leq 0.05$. The optimal levels (inflection point of the curve) of supplementation for the variables that responded quadratically were calculated as described by Sakomura & Rostagno (2016).

RESULTS

The levels of supplementation with fat-soluble vitamin induced a quadratic response ($p<0.05$) from FI, EP and EW and a linear response ($p \leq 0.05$) from EM. The animals which did not receive vitamin supplementation (0% inclusion) showed the worst results for FI, EP and EM (Table 2).

Table 2 – Performance and egg quality of white-egg laying hens from 28 to 44 days of age supplemented with different levels of fat-soluble vitamins.

Variable	Vitamin levels (%)							
	0.0	33.3	66.7	100.0	133.3	166.7		
Performance							*p* -value[14]	CV (%)[15]
FI[1] (g/bird/day)	76.58±1.10	89.24±1.00	90.66±1.59	91.16±1.50	91.40±1.70	92.40±1.23	<0.01[Q]	4.33
EP[2] (g/kg)	880.7±13.6	911.6±12.7	923.8±6.60	916.1±12.1	928.1±7.70	923.5±7.10	0.05[Q]	3.35
EW[3] (g)	58.46±0.29	59.39±0.48	57.88±0.35	58.16±0.49	58.58±0.48	60.13±0.48	<0.01[Q]	2.19
EM[4] (g)	51.454±0.89	54.16±1.09	53.43±0.39	53.27±0.92	54.35±0.66	55.40±0.49	<0.01[L]	4.04
CEM[5] (g/g)	1.511±0.03	1.676±0.04	1.705±0.04	1.720±0.03	1.682±0.02	1.678±0.02	<0.01[Q]	5.98
CDZ[6] (kg/dz)	1.060±0.02	1.199±0.02	1.265±0.04	1.295±0.03	1.186±0.02	1.230±0.03	<0.01[Q]	7.10
Egg quality								
YLK[7] (g/kg)	260.9±1.00	253.7±2.50	261.6±2.10	260.9±3.00	258.8±1.90	256.3±3.10	0.46	2.92
ALB[8] (g/kg)	654 ±1.00	658.90±3.60	647.8±2.00	649.8±3.60	649.8±2.10	656.0±2.60	0.04[Q]	3.38
SHL[9] (g/kg)	85.10±0.05	87.40±0.14	90.60±0.09	89.30±0.11	91.30±0.07	87.60±0.08	<0.01[Q]	1.29
HU[10]	84.31±0.94	84.38±0.95	84.48±0.94	83.61±0.54	84.88±1.04	84.48±0.99	0.84	3.45
SHLT[11] (mm)	0.303±0.006	0.330±0.004	0.334±0.004	0.327±0.005	0.332±0.002	0.325±0.003	<0.01[Q]	4.14
SHLS[12] (N)	3.265±0.11	3.720±0.09	3.779±0.07	3.832±0.10	3.924±0.05	3.613±0.05	<0.01[Q]	7.11
GRAV[13] (g/cm³)	1.081±0.0008	1.083±0.0010	1.084±0.0010	1.081±0.0008	1.083±0.0010	1.082±0.0008	0.17	0.23

[1]FI = feed intake, [2]EP = egg production, [3]EW = egg weight, [4]EM = egg mass, [5]CEM = feed conversion per egg mass, [6]CDZ = feed conversion per dozen eggs, [7]YLK = yolk content, [8]ALB = albumen content, [9]SHL= eggshell content, [10]HU = Haugh unit, [11]SHLT = eggshell thickness, [12]SHLS = eggshell strength, [13]GRAV = specific gravity.

[14] Q = quadratic response, L= linear response.

[15] CV = coefficient of variation.

The estimated optimal supplementation level for FI was 121.05%, corresponding to 9078.75, 2421 and 12.11 IU of vitamins A, D and E, respectively, and 2.18 mg of vitamin K per kilogram of diet (Table 3). For EP, the optimal level was 116.17%, representing 8712.75, 2323.4 and 11.62 IU of vitamins A, D and E, respectively, and 2.09 mg of vitamin K per kilogram of diet. The optimal supplementation level for EW, in turn, was 59.5%, which corresponds to 4462.5, 1190 and 5.95 IU of vitamins A, D and E, respectively, and 1.07 mg of vitamin K per kilogram of diet.

Feed conversion per egg mass and per dozen eggs responded quadratically to the increasing supplementation levels ($p \leq 0.05$). However, both variables were lowest in the group which received the treatment without inclusion of fat-soluble vitamins.

Table 3 – Regression equation and optimal levels of fat-soluble vitamin supplementation for white-egg laying hens from 28 to 44 days of age.

Variable	Quadratic/linear equation	R^2	Optimal level (%)[11]
Performance			
FI[1](g/bird/day)	$Y=78.68 + 0.2421X - 0.001X^2$	0.86	121.05
EP[2] (g/kg)	$Y=885.04 + 0.0697X - 0.0003X^2$	0.87	116.17
EW[3] (g)	$Y=58.966 - 0.0238X + 0.0002X^2$	0.55	59.50
EM[4] (g)	$Y= 52.225 + 0.0173X$	0.66	166.7
CEM[5] (g/g)	$Y=1.5338 + 0.0037X - 0.00002X^2$	0.88	92.50
CDZ[6] (kg/dz)	$Y=1.0759 + 0.0039X - 0.00002X^2$	0.79	97.50
Egg quality			
ALB[7] (g/kg)	$Y=656.8 - 0.0149X - 0.00008X^2$	0.37	93.13
SHL[8] (g/kg)	$Y=84.84 + 0.0113X - 0.00006X^2$	0.83	94.16
SHLT[9] (mm)	$Y=0.3075 + 0.0006X - 0.000003X^2$	0.80	100.0
SHLS[10] (N)	$Y=3.2746 + 0.0139X - 0.00007X^2$	0.76	99.66

[1]FI = feed intake, [2]EP = egg production, [3]EW = egg weight, [4]EM = egg mass, [5]CEM = feed conversion per egg mass, [6]CDZ = feed conversion per dozen eggs, [7]ALB = albumen content, [8]SHL = eggshell content, [9]SHLT = eggshell thickness, [10]SHLS = eggshell strength.

[11]Optimum level of the quadratic or linear equation.

The vitamin levels did not influence ($p>0.05$) YLK, HU or GRAV. Albumin, SHL, SHLT and SHLS, on the other hand, showed a quadratic response ($p\leq0.05$) to the treatments. The estimated optimal supplementation levels for the respective variables were 93.13%, 94.16%, 100% and 99.66%. These levels corresponded to the supplementation of 6984.75 IU, 1862.6 IU, 9.31 IU and 1.68 mg; 7062 IU, 1883.2 IU, 9.42 IU and 1.69 mg; 7500 IU, 2000 IU, 10 IU and 1.8 mg; and 7474.5 IU, 1993.2 IU, 9.96 IU and 1.79 mg of vitamins A, D, E and K per kilogram of diet, respectively.

DISCUSSION

The increasing levels of supplementation with fat-soluble vitamins resulted in improvements in FI, EP, EW and EM. Lin *et al.* (2002) observed a positive effect on the feed intake and egg-laying rate of birds which received diets supplemented with vitamin A when challenged with Newcastle virus and under thermal stress. Rodrigues *et al.* (2005) did not detect differences for EP, EW, FI and CEM using vitamin D at the levels of 1200, 2400 and 3600 IU/kg and 1200 and 2400 IU/kg in the pre- and post-laying phases, respectively. By contrast, Persia *et al.* (2013) observed positive effects on FI and EP in an experiment evaluating high levels of vitamin D supplementation.

The increasing supplementation levels elicited a reduction in CEM and CDZ. However, these variables showed their lowest values at the levels of 0% and 33%. This finding may be explained by the fact that feed conversion ratio is determined based on other variables (FI, EP or EM), which can interfere with results. Abawi & Sullivan (1989) found positive effects of increasing the level of supplementation with vitamins A, D and K on the feed conversion of broilers. Niu *et al.* (2009) observed an improvement in feed conversion at the vitamin E supplementation level of 100 mg/kg, in an experiment in which broilers received diets with 0, 100 or 200 mg/kg of the vitamin.

In this study, the worst results were observed in the group of hens fed diets without vitamin supplementation. This may be attributed to the induced vitamin deficiency, which possibly compromised the birds' performance. Compared to water-soluble vitamins, deficiencies caused by lack of fat-soluble vitamin supplementation take longer to appear because of the existing reserves in the liver and adipose tissue. However, approximately 6 to 8 weeks after the vitamins are withdrawn, the first deficiency symptoms begin to appear; e.g., weight loss, decreased egg-laying rate and decreased egg weight (Leeson & Summers, 2001).

Based on the presented results, increasing supplementation with fat-soluble vitamins influenced the eggshell variables (SHL, SHLT and SHLS). Calcium plays an important role in eggshell formation, constituting approximately 40% of it (Keshavarz, 2003). Layers are known to have vitamin D_3-dependent calcium-binding proteins that participate in the active transport of calcium in the intestinal membrane. These likely also act in the uterus, in the shell-forming gland (Wasserman and Taylor, 1968; Bar, 2008). Therefore, the SHL, SHLT and SHLS variables were likely influenced by the tested vitamin levels, given the importance of vitamin D_3 for calcium absorption.

The current results demonstrate that supplementing layer diets with vitamins is essential to achieving good results. We observed that part of the evaluated variables improved at supplementation levels close to or higher than that recommended by Rostagno *et al.* (2011) (represented herein by the level of 100%). Based on the optimal levels obtained for each variable, an average was calculated to determine the recommended level of fat-soluble vitamin supplementation, which was 109%. Other studies with vitamins have shown that dietary supplementation at levels higher than the recommended minimum allow birds to achieve their genetic potential (Barroeta *et al.*, 2012). Therefore, vitamin supplementation at minimum levels such as those recommended by the NRC (1994) appear not to be sufficient for birds to express their maximum genetic potential.

The best results for the evaluated variables were obtained at an average supplementation level of 109%. Therefore, the recommended values of fat-soluble vitamins to be supplemented in the diet of white-egg laying hens from 28 to 44 of age are 8175 IU of vitamin A, 2180 IU of vitamin D_3, and 10.9 IU of vitamin E and 1.96 mg of vitamin K per kilogram of diet.

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