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Cobalt and Vitamin B_{12} in Diets for Commercial Laying Hens on the Second Cycle of Production

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ABSTRACT

The supplementation of cobalt and vitamin B_{12} in diets for commercial laying hens on the second production cycle was studied. Four hundred and eighty light commercial laying hens, Lohmann LSL, were used at initial phase of forced molting laying period. The trial was conducted in a randomized design. The plots were the treatments which were constituted by combination of five cobalt levels (0.00; 0.30; 0.60; 0.90 and 1.20ppm) and two vitamin B_{12} levels (without and with 10µ/kg) and the split-plots were four periods (21, 42, 63 and 84 days) during the second period of production, with 4 repetitions and 12 hens per experimental unit. Food and water were provided *ad libitum* and eggs were collected twice daily. Performance and egg quality parameters were evaluated. At the end of experimental period, two layers from each treatment were slaughtered, and liver and blood samples were taken for analysis. Performance and egg quality were not different ($p>0.05$) among cobalt supplementation levels, although egg damage data were different ($p<0.05$). Supplementation with vitamin B_{12} decreased egg weight. No influence of cobalt or vitamin B_{12} supplementation was seen on the concentration of cobalt in the liver and yolk as well as on blood analysis (hematrocrit, hemoglobin, erythrocytes, and leukocytes). The results revealed that vitamin B_{12} supplementation was important for commercial laying hens on the second cycle of production, but not cobalt supplementation.

INTRODUCTION

Advances in husbandry, nutrition and breeding have improved the commercial production of laying hens. In spite of this progress, some nutritional aspects have not been fully understood yet. The trace of mineral cobalt, for instance, is not considered as an essential mineral for chickens, although it may be as much as 4% of the composition of the molecule of vitamin B_{12}. Literature concerning cobalt supplementation for chickens is scarce, particularly for laying hens. Some authors consider cobalt addition for laying hens unnecessary, and the mineral is then supplemented only as Vitamin B_{12} (National Research Council, NRC, 1994). According to Rostagno *et al.* (2000), 0.2 ppm of cobalt in the diet may be used for laying hens. However, there is no indication that this trace mineral is unnecessary for laying hens. Birds synthesize vitamin B_{12} using cobalt inside the ceca, but the levels are below the requirements, and it must be supplemented (McDonald *et al.*, 1975). Furthermore, there is no consensus about cobalt supplementation in chicken diets. In practice, the industries of mineral supplement add, on an average, 0.29 g of cobalt per ton of feed. Considering the high price of cobalt, adding it may represent an additional cost for poultry production. Besides, environmental issues have also to be considered, since cobalt may become a pollutant if used inadequately or when it is not really needed.

In order to produce technical information to this inquiry, the present work was carried out to study the need of supplementing cobalt and vitamin B_{12} in diets of laying hens during the cycle of egg production .

MATERIAL AND METHODS

Conventional cages with trough feeder and nipple drinker were used, with 12 laying hens per cage. Lohmann LSL laying hens (480 birds) at the beginning of the second cycle of production were used. Experimental phase started at 62.8% of egg production. Lights were on from 3am to 8pm, with 17L:7D. Water and food were given *ad libitum* and eggs were collected twice daily, at 10am and at 4pm. Environment temperature was registered with a thermometer placed in the middle of the poultry house. The maximum, minimum and average temperatures were 26.2, 13.3 and 19.7ºC, respectively. Ten diets were produced by the combination of five levels of cobalt (0.00; 0.30; 0.60; 0.90 and 1.20 ppm) and two levels of vitamin B_{12} (0 and 10 µg/kg). The control treatment was given a basal corn-soybean diet, with no supplementation of cobalt and vitamin B_{12} (Table 1). Cobalt and vitamin B_{12} were supplemented by addition of pre-mixtures, as described in Table 2.

A split plot experimental design was used with four periods (21, 42, 63 and 84 days) and four repetitions. Forty cages were used with 12 birds in each. A factorial schedule 5x2 was used, with 5 cobalt supplementation levels, with and without vitamin B_{12} supplementation.

Performance was evaluated by egg production (%/hen/day), egg loss (%/hen/day), egg weight (g), egg mass (g), feed intake (g/hen/day) and feed conversion (g/g). Egg quality was evaluated in the last three days of each period using specific weight (g/cm^3), shell percentage (%), shell thickness (mm), weight of the shell per unit of surface of area (mg/cm^3) – WSUSA– (Abdallah *et al.*, 1993) and internal quality was expressed in Haugh units (Card & Nesheim, 1968).

At the end of the experiment, one hen was sacrificed per cage thirty minutes after laying an egg, and a sample from liver tissue was collected. The egg was collected for yolk analysis and stored at 5ºC for later processing (lyophilized and degreased). Cobalt concentration was determined in the yolk and liver (dry matter basis) using flame atomic spectrometry.

Two hens per treatment were killed 30 minutes after laying and blood samples were taken to determine hematocrit, hemoglobin, erythrocyte and leukocyte numbers.

The data were submitted to statistical analysis using the software SISVAR - Variance Analysis System for Balanced Data (Ferreira, 1999).

Table 1 – Composition of the basal diet used in the experiment.

Ingredients	kg/ton
Corn	611.50
Soybean meal	146.01
Gluten meal 60	44.46
Wheat meal	80.43
Limestone	92.01
Dicalcium phosphate	14.09
Salt, iodized	3.00
Soybean oil	5.00
DL-methionine	0.50
Vitamin supplement[1]	1.00
Mineral supplement[2]	1.00
Inert	1.00
Total	1,000.00
Calculated composition	
Metabolizable energy (kcal/kg)	2,750
Crude Protein (%)	15.94
Methionine (%)	0.312
Methionine + Cystine (%)	0.586
Lysine (%)	0.782
Calcium (%)	3.904
Available phosphorus (%)[3]	0.361
Cobalt (ppm)	0.065

1 - Vitamin supplement, levels per kg: Vitamin A, 8,000,000 IU; Vitamin D_3, 2,000,000 IU; Vitamin E, 15g; Vitamin K_3, 3g; Thiamin, 1g; Riboflavin, 4.08g; Pyridoxine, 1g; Pantothenic acid, 5.5g; Folacin, 2g; Nicotinamide, 19g; Antioxidant, 10g; Selenium, 0,25g.
2 - Mineral supplement, levels per kg: Fe, 20g; Cu, 4g; Mn, 75g; Zn, 50g; I, 1.5g.
3 - One third of vegetal phosphorus was available.

Table 2 – Cobalt and vitamin B_{12} supplemention to the experimental diets.

Ingredient (g)	Vit.B_{12} (µg/kg)	Cobalt (ppm)				
		0.00	0.30	0.60	0.90	1.20
Premix Co 2400ppm[1]	0	0.0	12.5	25.0	37.5	50.0
Inert q.s.p.		100.0	87.5	75.0	62.5	50.0
Total (g)		100.0	100.0	100.0	100.0	100.0
Premix Co 2400ppm[1]	10	0.0	12.5	25.0	37.5	50.0
Premix Vit.B_{12} 20ppm[2]		50.0	50.0	50.0	50.0	50.0
Inert q.s.p.		50.0	37.5	25.0	12.5	0.0
Total (g)		100.0	100.0	100.0	100.0	100.0

1 - 12g $CoSO_4.7H_2O$ (20%) per kg.
2 - 20g Vitamin B_{12} (0.1%) per kg.

Table 3 – Effect of cobalt supplementation on performance.

Performance	Cobalt (ppm)						
	0.00	0.30	0.60	0.90	1.20	Mean	CV(%)
Egg production (%/hen/day)	82.9	85.9	79.4	83.6	83.7	83.1	12.56
Egg loss (%/hen/day)[1]	4.30ab	3.78b	6.23[a]	4.23ab	5.31ab	4.77	64.40
Egg weight (g)	69.5	68.8	69.1	68.6	69.8	69.2	3.76
Egg mass (g)	57.6	59.1	54.9	57.4	58.4	57.5	13.61
Feed intake (g)	121.4	122.2	119.6	120.4	121.4	121.0	5.14
Feed conversion (g/g)	2.11	2.07	2.18	2.10	2.08	2.11	11.36

1 - Means followed by different letters in the row are statistically different ($p<0.05$) by SNK test.

RESULTS AND DISCUSSION

Performance

Cobalt supplementation had no effect ($p>0.05$) on performance, except for egg loss (Table3). No significant interaction ($p>0.05$) was seen between cobalt and vitamin B_{12} supplementation in the evaluated performance characteristics, demonstrating that the effects of vitamin B_{12} and cobalt supplementation were independent.

Vitamin B_{12} had no effect (Table 4) on egg production ($p>0.05$), probably due to the relatively short experimental period (84 days), and the absence supplementation of vitamin B_{12} in the diet did not result in clinical signs related to vitamin deficiency. Squires & Naber (1992) reported a decrease in egg production only after 12 weeks of production when a diet without B_{12} was given. As reported by Scott et al. (1982) birds have hepatic storage of vitamin B_{12} and the reserves are not affected up to 12 weeks when that nutrient is not given in the diet.

Egg loss was affected ($p<0.05$) by cobalt levels (Table 3), which could not be explained by regression analysis, but was not influenced ($p>0.05$) by the absence or presence of vitamin B_{12}.

Vitamin B_{12} increased ($p<0.01$) egg weight, data similar to those were reported by Skinner *et al.* (1951) and Squires & Naber (1992), which demonstrated the importance of vitamin B_{12} supplementation. Egg mass, feed intake and feed conversion were not affected ($p>0.05$) by vitamin B_{12} supplementation.

Table 4 – Effect of vitamin B_{12} on performance.

Performance	Vitamin B_{12} (µg/kg)			
	0.00	10.00	Mean	CV(%)
Egg production (%/hen/day)	83.4	82.9	83.1	12.56
Egg loss (%/hen/day)[1]	4.83	4.72	4.77	64.40
Egg weight (g)	68.6	69.8	69.2	3.76
Egg mass (g)	57.2	57.8	57.5	13.61
Feed intake (g)	121.3	120.8	121.0	5.14
Feed conversion (g/g)	2.12	2.09	2.11	11.36

1 - No difference was observed among treatments ($p>0.05$) by SNK test.

Table 5 – Cobalt effect on egg quality during the experimental period.

Egg quality	Cobalt (ppm)[1]						
	0.00	0.30	0.60	0.90	1.20	Mean	CV(%)
Specific weight (g/cm^3)	1.0783	1.0777	1.0772	1.0781	1.0772	1.0777	0.17
Egg shell percentage (%)	8.83	8.82	8.73	8.76	8.68	8.76	4.46
Egg shell thickness (mm)	365.0	364.4	361.9	362.9	359.7	362.8	4.78
WSUSA (mg/cm^3)[2]	77.1	76.9	76.1	76.4	75.8	76.4	4.40
Haugh unit	92.1	92.5	92.0	92.2	91.6	92.1	4.28

1 - Regression analysis ($p>0.05$).
2 - WSUSA – weight of shell per unit of surface area.

Table 6 – Vitamin B_{12} effect on egg quality during the experimental period.

Egg quality	Vitamin B_{12} (µg/kg)			
	0.00	10.00	Mean	CV(%)
Specific weight (g/cm^3)[1]	1.0786 a	1.0767 b	1.0777	0.17
Egg shell percentage (%)	8.88 a	8.65 b	8.76	4.46
Egg shell thickness (mm)	367.2 a	358.4 b	362.8	4.78
WSUSA (mg/cm^3)[2]	77.2 a	75.8 b	76.4	4.40
Haugh unit	92.3	91.9	92.1	4.28

1 - Means followed by different letters in the row are statistically different ($p<0.05$) by F test.
2 - WSUSA weight of shell per unit of surface area.

Egg quality

Cobalt supplementation had no effect (p>0.05) on egg quality (Table 5). This indicates that cobalt supplementation is not necessary in order to improve internal and external egg quality. There was no interaction (p>0.05) between supplementation of cobalt and supplementation of vitamin B_{12} on egg quality parameters.

Vitamin B_{12} supplementation decreased (p<0.01) specific egg weight when compared to the treatment without vitamin B_{12}. Vitamin B_{12} increased egg size and, consequently, specific weight was decreased. Eggs were smaller when no vitamin B_{12} was added and specific weight was inversely proportional to the size of the egg. Smaller shell percentage (p<0.01) was observed with vitamin B_{12} supplementation. In the absence of vitamin B_{12}, egg shell thickness and the weight of the egg shell per unit of surface of area (WSUSA) were higher (p<0.01) than the treatment with vitamin B_{12} (Table 6). This finding, as it was already expected, was inversely proportional to the weight of the eggs (Squires & Naber, 1992). The same was observed for egg shell percentage, egg shell weight and thickness.

The treatments had no effect (p>0.05) on Haugh unit values. Maybe the trial period was too short to induce any change in the internal quality of the eggs.

Cobalt concentration in the liver and the yolk

Cobalt concentration (dry matter basis) in the liver or in the yolk was not affected (p > 0.05) by the treatments (Table 7). This finding could be explained by the low level of cobalt used in this study when compared to those used by Southern & Baker (1980), who observed 0.03 to 0.85 ppm of cobalt in dry matter basis when no cobalt was used, and 16 to 55.5 ppm when 250 ppm of cobalt was supplemented. Adding vitamin B_{12} to the diet had no effect (p>0.05) on cobalt concentration in the liver and yolk.

Table 7 – Effect of cobalt and vitamin B_{12} supplementation on cobalt levels in the liver and yolk (dry matter basis).

Cobalt supplementation (ppm)[1]	Cobalt (ppm, DM basis) Liver	Yolk
0.00	0.1875	0.0877
0.30	0.1825	0.0942
0.60	0.1750	0.1007
0.90	0.1875	0.0877
1.20	0.1950	0.0942
Vitamin B_{12} supplementation (µg/kg)[2]		
0.00	0.1860	0.0916
10.00	0.1850	0.0942

1- Regression analysis (p>0.05).
2 - No difference was verified among treatments (p>0.05) by F test.

Blood analysis

The addition of cobalt plus vitamin B_{12} in the diet had no effect (p>0.05) on hematocrit, hemoglobin, erythrocytes and leukocyte numbers (Table 8), and the supplementation of cobalt only also did not interfere

Table 8 – Effect of cobalt and vitamin B_{12} on hematocrit, hemoglobin, erythrocyte and leukocyte values.

Cobalt (ppm)[1]	Hematocrit (%)	Hemoglobin (g/dL)	Erythrocytes ($x10^3$ cells/mm^3)	Leukocytes (cells/mm^3)
0.00	37.75	17.70	2.755	717,600
0.30	35.37	15.95	2.705	688,650
0.60	37.25	17.17	2.823	762,400
0.90	36.75	17.17	2.760	826,400
1.20	37.25	17.05	2.715	697,600
Vit. B_{12} (µg/kg)[2]				
0.00	37.10	16.73	2.787	745,920
10.00	36.65	17.29	2.716	731,140

1 - Regression analysis (p>0.05).
2 - Not statistically different (p > 0.05) by F test.

with erythrocyte and hemoglobin values ($p > 0.05$). Conversely, Diaz *et al.* (1994) reported an increase in hemoglobin and erythrocytes in chickens fed with diet containing cobalt.

CONCLUSIONS

Cobalt supplementation in the diets of laying hens in the second cycle of production did not influence egg production, egg quality, blood characteristics, and cobalt levels in the liver and yolk within the trial period, suggesting that there is no need for cobalt supplementation; however, vitamin B_{12} supplementation increased egg weight.

REFERENCES

Abdallah AG, Harms RH, El-Husseiny O. Various methods of measuring shell quality in relation to percentage of cracked eggs. Poultry Science 1993; 72(11):2038-2043.

Card LE, Neshein MC. Produccion Avícola. New York: Ithaca, 1968. 392p.

Diaz GJ, Julian RJ, Squires EJ. Cobalt-induced polycythaemia causing right ventricular hypertrophy and ascites in meat-type chickens. Avian Pathology 1994; 23 (1):91-104.

Ferreira DF. Sistema para análise de variância para dados balanceados (SISVAR). Lavras: UFLA; 1999. 92p.

McDonald P, Edwards RA, Greenhalgh JFD. Minerais . In: Nutrición animal. 2.ed. Zaragoza: Acribia, 1975. p.107-109.

National Research Council. Nutrient Requirements of Poultry. 9.ed. Washington, 1994. 155p.

Rostagno HS, Albino LFT, Donzele JL, Gomes PC, Ferreira AS, Oliveira RF, Lopes DC. Tabelas Brasileiras para aves e suínos; Composição de alimentos e exigências nutricionais. Viçosa: UFV. Imprensa Universitária, 2000. 141p.

Scott ML, Neshein MG, Young RJ. The vitamins. In: ML Scott (editor). Nutrition of the chickens. 3.ed. New York, 1982. p.237-243.

Skinner JL, Quisenberry JH, Couch JR. High efficiency and APF concentrates in the ration of the laying fowl. Poultry Science 1951; 30(3): 319-324.

Southern LL, Baker DH. The effect of methionine or cystine on cobalt toxicity in the chick. Poultry Science 1980; 60(7): 1303-1308.

Squires MW, Naber EC. Vitamin profiles of eggs as indicators of nutritional status in the laying hen: Vitamin B_{12} study. Poultry Science 1992; 71(12): 2075-2082.