



## Cobalt and Vitamin B<sub>12</sub> in Diets for Commercial Laying Hens on the Second Cycle of Production

### ■ Author(s)

Kato RK<sup>1</sup>  
Bertechini AG<sup>2</sup>  
Fassani EJ<sup>1</sup>  
Santos CD<sup>3</sup>  
Dionizio MA<sup>4</sup>  
Fialho ET<sup>2</sup>

1-Ph.D student – Depto. de Zootecnia - UFLA/  
CAPES, Lavras - MG - Brasil

2-Prof<sup>o</sup> do Depto. de Zootecnia - UFLA/CAPES,  
Lavras - MG - Brasil

3-Prof<sup>o</sup> do Depto. de Química - UFLA, Lavras -  
MG - Brasil

4-Ph.D student – Depto. de Zootecnia – UFV/  
CAPES, Viçosa - MG - Brasil

### ■ Mail Address

Antonio Gilberto Bertechini

Depto. de Zootecnia - Universidade Federal de Lavras  
Caixa Postal 37  
37200-000 - Lavras - MG - Brasil

E-mail: bertechi@ufla.br

### ■ Keywords

cobalt, egg production, laying hens, nutrition,  
vitamin B<sub>12</sub>

### ABSTRACT

The supplementation of cobalt and vitamin B<sub>12</sub> 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<sub>12</sub> 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<sub>12</sub> decreased egg weight. No influence of cobalt or vitamin B<sub>12</sub> supplementation was seen on the concentration of cobalt in the liver and yolk as well as on blood analysis (hematocrit, hemoglobin, erythrocytes, and leukocytes). The results revealed that vitamin B<sub>12</sub> 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<sub>12</sub>. 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<sub>12</sub> (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<sub>12</sub> 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

Arrived: May 2002  
Approved: September 2002



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<sub>12</sub> 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 19.7°C, 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<sub>12</sub> (0 and 10 µg/kg). The control treatment was given a basal corn-soybean diet, with no supplementation of cobalt and vitamin B<sub>12</sub> (Table 1). Cobalt and vitamin B<sub>12</sub> 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<sub>12</sub> 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<sup>3</sup>), shell percentage (%), shell thickness (mm), weight of the shell per unit of surface of area (mg/cm<sup>2</sup>) – 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 5°C for later processing (lyophilized and degreased). Cobalt

**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 <sup>1</sup>	1.00
Mineral supplement <sup>2</sup>	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 (%) <sup>3</sup>	0.361
Cobalt (ppm)	0.065

1 - Vitamin supplement, levels per kg: Vitamin A, 8,000,000 IU; Vitamin D<sub>3</sub>, 2,000,000 IU; Vitamin E, 15g; Vitamin K<sub>3</sub>, 3g; Thiamin, 1g; Riboflavin, 4.08g; Pyridoxine, 1g; Pantothenic acid, 5.5g; Folicin, 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.

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 2** – Cobalt and vitamin B<sub>12</sub> supplementation to the experimental diets.

Ingredient (g)	Vit.B <sub>12</sub> (µg/kg)	Cobalt (ppm)				
		0.00	0.30	0.60	0.90	1.20
Premix Co 2400ppm <sup>1</sup>	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 <sup>1</sup>	10	0.0	12.5	25.0	37.5	50.0
Premix Vit.B <sub>12</sub> 20ppm <sup>2</sup>		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<sub>4</sub>·7H<sub>2</sub>O (20%) per kg.

2 - 20g Vitamin B<sub>12</sub> (0.1%) per kg.

**Table 3** – Effect of cobalt supplementation on performance.

Performance	Cobalt (ppm)					Mean	CV(%)
	0.00	0.30	0.60	0.90	1.20		
Egg production (%/hen/day)	82.9	85.9	79.4	83.6	83.7	83.1	12.56
Egg loss (%/hen/day) <sup>1</sup>	4.30ab	3.78b	6.23 <sup>a</sup>	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 (Table 3). No significant interaction (p>0.05) was seen between cobalt and vitamin B<sub>12</sub> supplementation in the evaluated performance characteristics, demonstrating that the effects of vitamin B<sub>12</sub> and cobalt supplementation were independent.

Vitamin B<sub>12</sub> 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<sub>12</sub> 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<sub>12</sub> was given. As reported by Scott et al. (1982) birds have hepatic storage of vitamin B<sub>12</sub> 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<sub>12</sub>.

Vitamin B<sub>12</sub> 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<sub>12</sub> supplementation. Egg mass, feed intake and feed conversion were not affected (p>0.05) by vitamin B<sub>12</sub> supplementation.



**Table 4** – Effect of vitamin B<sub>12</sub> on performance.

Performance	Vitamin B <sub>12</sub> (µg/kg)			
	0.00	10.00	Mean	CV(%)
Egg production (%/hen/day)	83.4	82.9	83.1	12.56
Egg loss (%/hen/day) <sup>1</sup>	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) <sup>1</sup>					Mean	CV(%)
	0.00	0.30	0.60	0.90	1.20		
Specific weight (g/cm <sup>3</sup> )	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 <sup>3</sup> ) <sup>2</sup>	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<sub>12</sub> effect on egg quality during the experimental period.

Egg quality	Vitamin B <sub>12</sub> (µg/kg)			
	0.00	10.00	Mean	CV(%)
Specific weight (g/cm <sup>3</sup> ) <sup>1</sup>	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 <sup>3</sup> ) <sup>2</sup>	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<sub>12</sub> on egg quality parameters.

Vitamin B<sub>12</sub> supplementation decreased ( $p < 0.01$ ) specific egg weight when compared to the treatment without vitamin B<sub>12</sub>. Vitamin B<sub>12</sub> increased egg size and, consequently, specific weight was decreased. Eggs were smaller when no vitamin B<sub>12</sub> 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<sub>12</sub> supplementation. In the absence of vitamin B<sub>12</sub>, 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<sub>12</sub> (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

**Table 7** – Effect of cobalt and vitamin B<sub>12</sub> supplementation on cobalt levels in the liver and yolk (dry matter basis).

Cobalt supplementation (ppm) <sup>1</sup>	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 <sub>12</sub> supplementation (µg/kg) <sup>2</sup>		
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.

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<sub>12</sub> to the diet had no effect ( $p > 0.05$ ) on cobalt concentration in the liver and yolk.

## Blood analysis

The addition of cobalt plus vitamin B<sub>12</sub> 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<sub>12</sub> on hematocrit, hemoglobin, erythrocyte and leukocyte values.

Cobalt (ppm) <sup>1</sup>	Hematocrit	Hemoglobin	Erythrocytes	Leukocytes
	(%)	(g/dL)	(x10 <sup>3</sup> cells/mm <sup>3</sup> )	(cells/mm <sup>3</sup> )
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 <sub>12</sub> (µg/kg) <sup>2</sup>				
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<sub>12</sub> supplementation increased egg weight.

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