Influence of Vitamin B_{12} and Cobalt on performance of laying hens

Ingrid Halle and Mohammad Ebrahem*

Summary

The effect of Vitamin B_{12} and Cobalt (Co) on laying performance, egg composition, and organ mass and haematocrit was studied in laying hens. A total of 216 laying hens were allocated to 8 dietary treatment groups (0/5/10/20 µg vitamin B_{12} per kg, 0.6 mg Co, 0.6 mg Co + 5 µg B_{12}, 0.6 mg Co + 10 µg B_{12}, 0.6 mg Co + 20 µg B_{12}) and fed for 13 laying months (364 days). The daily feed intake per hen was significantly increased in the mean value of the total laying period of the 5 µg B_{12}-Group compared to the other B_{12}-Groups and the Control. The laying intensity was ranged between 90 to 93 % and was not different between groups. The egg weight and the daily egg mass production were significantly increased with supplementation of vitamin B_{12} (all groups) and B_{12} x Co. Feed conversion was significantly improved in the group with 5 µg B_{12} per kg feed and also the combination B_{12} x Co. The supplementation of 5 to 20 µg B_{12} to the diet significantly increased the final hen body weight. Co alone without B_{12} in the diet significantly increased the mass of the eggs and did not change the other laying parameters. Supplementation of diets with vitamin B_{12} or Co or both additives didn't directly improve the egg composition and the yolk colour. The results of slaughtering showed that supplementation of 20 µg B_{12} the liver mass decreased significantly and the haematocrit content increased significantly compared to the Control and the 0.6 mg Co-Group. The supplementation of 0.6 mg Co significantly increased the spleen mass. The results allow the conclusion that a supplementation of 5 µg vitamin B_{12} per kg laying hen feed was sufficient to compensate the deficiencies on feed intake and laying performance. An additional supplementation of Co to feed does not have additional advantages for the birds.

Keywords: Vitamin B_{12}, Cobalt, laying hen, laying performance, egg composition, haematocrit

Zusammenfassung

Einfluss von Vitamin B_{12} und Kobalt auf die Leistungsentwicklung von Legehennen

Der Einfluss der Supplementierung von Vitamin B_{12} und Kobalt (Co) auf die Leistungsentwicklung, die Eizusammensetzung, die Organgewichte und den Anteil an Hämatokrit am Volumen des Blutes wurde an Legehennen untersucht. Im Versuch über 13 Legemonate (364 Tage) wurden 216 Hennen (LB) in 8 Versuchsgruppen (0/5/10/20 µg Vitamin B_{12} per kg, 0,6 mg Co, 0,6 mg Co + 5 µg B_{12}, 0,6 mg Co + 10 µg B_{12}, 0,6 mg Co + 20 µg B_{12}) eingeteilt. Der tägliche Futterverzehr der Hennen der Gruppe mit 5 µg Vitamin B_{12} per kg war signifikant höher im Vergleich zu den weiteren B_{12}-Gruppen und der Kontrolle. Die Legeintensität von 90 bis 93 % war zwischen den Gruppen gleich. Sowohl das Eigewicht als auch die tägliche Eimasseproduktion verbesserten sich statistisch gesichert durch die Supplementierung von Vitamin B_{12} oder der Kombination aus B_{12} + Co ins Futter. Die Zugabe von 5 bis 20 µg Vitamin B_{12} hatte einen signifikant positiven Einfluss auf die Lebendmasse am Versuchsende. Die alleinige Zugabe von Co ins Futter erhöhte gesichert das Eigewicht und veränderte nicht die weiteren Legeparameter. Die Eizusammensetzung und die Dotterfarbe wurden weder durch die Zugabe von B_{12} noch Co verändert. Die Ergebnisse der Ausschlachtung zeigten, dass durch die Zugabe von 20 µg Vitamin B_{12} das Lebergewicht signifikant reduziert und der Gehalt an Hämatokrit gesichert höher war im Vergleich zu Hennen der Kontrolle und der 0,6 mg Co Gruppe. Die Supplementierung von 0,6 mg Co erhöht signifikant das Gewicht der Milz. Die Ergebnisse lassen die Schlussfolgerung zu, dass eine Supplementierung von 5 µg Vitamin B_{12} pro kg Hennenfutter ausreichend war und eine zusätzliche Co Supplementierung keinen additiven Einfluss hat.

Schlüsselworte: Vitamin B_{12}, Kobalt, Legehenne, Legeleistung, Eizusammensetzung, Hämatokrit

* Friedrich-Loeffler-Institute, Federal Research Institute for Animal Health, Institute of Animal Nutrition, Bundesallee 50, Braunschweig, Germany
Corresponding author: PD Dr. Ingrid Halle, E.mail: ingrid.halle@fli.bund.de

Vitamin B_{12} belongs to a specific group of Cobalt containing corrinoids with biological activity in humans and animals. The term vitamin B_{12} is restricted to recommended biochemical nomenclature for a form of cobalamin. Cobalamin does not occur in plants, it is synthesized in nature by certain bacteria, fungi and algae (Green, 2005). Microbes in the rumen of ruminants incorporate Co into a corrin ring to form vitamin B_{12}. Poultry obtain their B_{12} either pre-formed in feed or indirectly by ingesting faeces. Vitamin B_{12} is available commercially as cyanocobalamin for addition to feed. Vitamin B_{12} is an essential component of several enzyme systems that carry out a number of very basic metabolic functions in the animal's body (McDowell, 1989). This vitamin plays a central role in normal functions of the brain and nervous system, in the homocystein metabolism, in the blood function, energy metabolism, cell division and function of the immune system (EFSA 2009 a, 2010). 4 µg per kg feed are indicated by the NRC (1994) and 9 µg B_{12} per kg by the GfE (1999) as the requirement of laying hens.

There is no evidence that monogastric animals (pigs and poultry) require Cobalt (Co) other than through vitamin B_{12}. Consequently, there is no need for any Co supplementation to the feed for these animals (EFSA, 2009 b). Although Co is not necessary for the nutrition of laying hens, it is often added into the vitamin-mineral premix.

The objective of this study was to determine the effect of a Vitamin B_{12}-free diet supplemented with Vitamin B_{12} or/and Cobalt on laying hen performance.

Material and Methods

A total of 216 laying hens (LB) were used in the feeding study. The hens were allocated to 8 groups of 26 hens each (Table 1). The hens were kept individually in a cage battery. The study commenced when the hens were 22 weeks old and continued until the 13th laying month. The basal diet was Vitamin B_{12}-free and contained Co from only natural sources (wheat, barley, corn, soya bean meal). The microbiological analyses (LUFA Speyer, LUFA SP 22013) of the control diet yielded a value of 8 µg B_{12} per kg. This value resulted from microbiological contamination of the feedstuffs. The basal diet was supplemented with 0/5/10/20 µg B_{12} per kg, 0.6 mg Co, 0.6 mg Co + 5 µg B_{12}, 0.6 mg Co + 10 µg B_{12} and 0.6 mg Co + 20 µg B_{12} per kg (Table 2). Each hen was offered the respective diet and water ad libitum. The number of laid eggs was recorded daily and the individual feed consumption was recorded monthly per hen. Each month the collected eggs were weighed four times within two weeks. In the 3rd, 6th and 10th laying month all eggs laid over 3 consecutive days were collected and the yolk weight, albumen weight and shell weight were measured. The color of the egg yolk was examined with the Roche-Fan. Body weight of hens was recorded at the start and at the end of the trial.

Table 1:
Trial design

Group	B_{12} µg/kg	Co mg/kg
1	0	0
2	5	0
3	10	0
4	20	0
5	0	0.6
6	5	0.6
7	10	0.6
8	20	0.6

Table 2:
Ingredient composition, analysed and calculated nutrients in the diet (g/kg)

Ingredient	Diet
Wheat	547.0
Barley	100.0
Corn	50.0
Soya bean meal	175.0
Soya oil	20.0
Di-calcium-phosphate	12.7
Calcium carbonate	79.5
Sodium chloride	3.3
DL-Methionine	1.1
L-Lysine-HCl	1.4
Premix [1)]	10.0
Vitamin $_{B12}$ [2)], µg/kg	0/5/10/20
Co [2)], mg/kg	0/0.6
Dry matter [3)]	894
Crude protein [3)]	161
ME, MJ/kg [4)]	11.36
Lysine [5)]	8.5
Methionine + Cystine [5)]	6.6
Cobalt, µg/kg [6)]	34
Vitamin B_{12}, µg/kg [7)]	8

[1)] Vitamin- mineral premix provided per kg of diet: Fe, 40 mg; Cu, 10 mg; Zn, 80 mg; Mn, 100 mg; Se, 0.25 mg; I, 1.2 mg; vitamin A, 10000 IE, vitamin D_3, 2500 IE; vitamin E, 20 mg; vitamin K_3, 4 mg; thiamine, 2.5 mg; riboflavin, 7 mg; pyridoxine, 4 mg; nicotinic acid, 40 mg; pantothenic acid, 10 mg; folic acid, 0.6 mg; biotin, 25 µg; choline chloride, 400 mg
[2)] Cobalt carbonat – 5 % Co; Cyanocobalamin Product – 0.1 % vitamin B_{12}
[3)] Analysed values
[4)] Calculated values (WPSA; 1985)
[5)] Calculated values
[6)] Control group calculated values (Souci et al., 2008)
[7)] Control group analysed values

Eight hens of the Control, 20 µg B_{12}-Group, 0.6 mg Co-Group and 0.6 mg Co + 20 µg B_{12}-Group each were slaughtered at the end of the trial to determine hematocrit, expressed as a percentage of the total blood volume and organ weights. Blood was collected into heparinized tubes from the neck vessels of the hens. Hematocrit was determined by using heparinized capillaries for blood sampling after 8 minutes of centrifugation at 13,000 RPM in a micro-hematocrit centrifuge.

Data were analyzed with a two-way ANOVA: yijk = µ + Vi + Cj + VCij + eijk, with yijk = observation, µ = general mean, Vi = vitamin B_{12} (0, 5, 10 and 20 µg/kg), Cj = Cobalt (0 and 0.6 mg/kg), interaction VCij and eijk = error term (random). Multiple comparisons of means were carried out using the Student-Newman-Keuls Test ($P \leq 0.05$). The statistical analyses were performed by the SAS software package (Version 9.1).

Results

In total 5 hens died apparently without relevance to the treatment. The laying performance of the hens is shown in Table 3, 4 and 5. The daily feed intake per hen was significantly increased ($P \leq 0.05$) over the total laying period of the 5 µg B_{12}-Group compared to the other B_{12}-Groups and the Control (Table 4). The laying intensity of the hens was range between 90 % and 93 % in mean of the 13th laying month and was not different between groups. The egg weight and the daily egg mass production were significantly increased with supplementation of vitamin B_{12} (all groups) and B_{12} x Co in the feed. Feed conversion was significantly improved in the group with 5 µg B_{12} per kg feed and also the combination B_{12} x Co. The supplementation of 5 µg to 20 µg B_{12} to the diet significantly increased the final body weight of hens (Table 4). Co alone without vitamin B_{12} in the diet significantly increased the mass of the eggs and did not change the other laying parameters. Supplementation of diets with vitamin B_{12} or Co or both additives didn't directly improve the egg composition or the yolk colour (Table 6). The results of slaughtering showed that with supplementation of 20 µg B_{12} the liver mass significantly decreased and the haematocrit significantly increased compared to the Control and 0.6 mg Co-Group (Table 7). The supplementation of 0.6 mg Co significantly increased the spleen mass.

Table 3:
Performance of laying hens (Means, SD, P-value)

Treatments		Feed intake g/hen/day	Laying intensity %	Egg weight g/egg	Egg mass production g/hen/day	Feed : egg mass production kg/kg	Body weight, g/hen	
B_{12} µg/kg	Co mg/kg						Start	End
0	0	109.4 ± 12.8	92.2 ± 8.9	61.9 ± 4.8	57.2 ± 7.1	1.923 ± 0.17	1637 ± 133	1741 ± 226
5	0	109.2 ± 9.2	93.0 ± 8.6	62.9 ± 5.2	58.3 ± 6.0	1.883 ± 0.17	1637 ± 114	1845 ± 125
10	0	111.5 ± 10.9	92.2 ± 10.4	63.0 ± 6.5	58.0 ± 8.0	1.948 ± 0.25	1637 ± 137	1884 ± 193
20	0	110.7 ± 12.6	91.9 ± 9.8	62.3 ± 5.7	57.0 ± 6.9	1.961 ± 0.30	1637 ± 185	1922 ± 255
0	0.6	108.1 ± 12.7	90.3 ± 11.5	61.9 ± 4.4	55.7 ± 7.4	1.963 ± 0.29	1637 ± 148	1778 ± 164
5	0.6	109.4 ± 12.2	91.9 ± 9.0	63.4 ± 6.2	58.1 ± 6.8	1.893 ± 0.17	1637 ± 134	1828 ± 168
10	0.6	112.5 ± 9.6	92.0 ± 10.5	63.9 ± 5.4	58.7 ± 7.5	1.947 ± 0.30	1637 ± 122	1876 ± 160
20	0.6	109.0 ± 9.6	92.6 ± 11.0	63.2 ± 4.7	58.6 ± 7.9	1.892 ± 0.32	1637 ± 111	1878 ± 138
ANOVA, P-value								
B_{12}		0.001	0.13	0.001	0.001	0.001	1.0	0.001
Co		0.31	0.12	0.006	0.63	0.62	1.0	0.76
B_{12} x Co		0.10	0.059	0.34	0.001	0.001	1.0	0.73

Table 4:
Influence of vitamin B_{12} on performance of laying hens (Means)

Vitamin B_{12}, µg/kg	0	5	10	20
Feed intake, g/hen/day	108.8 b	109.3 b	112.0 a	109.8 b
Laying intensity, %	91.3	92.4	92.1	92.3
Egg weight, g/egg	61.9 b	63.1 a	63.5 a	62.8 a
Egg mass production, g/hen/day	56.5 b	58.2 a	58.3 a	57.8 a
Feed : egg mass production, kg/kg	1.943 a	1.888 b	1.947 a	1.927 a
Final body weight, g/hen	1759 b	1836 a	1880 a	1900 a

Table 5:
Influence of Co on performance of laying hens (Means)

Co, mg/kg	0	0.60
Feed intake, g/hen/day	110.2	109.7
Laying intensity, %	92.3	91.7
Egg weight, g/egg	62.5 b	63.1 a
Egg mass production, g/hen/day	57.6	57.8
Feed to egg mass production, kg/kg	1.928	1.924
Final body weight, g/hen	1847	1839

Discussion

The effect of Vitamin B_{12} and Cobalt on laying performance, egg composition, and organ mass and haematocrit was studied in laying hens. The available data on the requirement of Vitamin B_{12} in laying hens is very limited.

In the present study the main sign of prolonged vitamin B_{12} deficiency in laying hens was a reduced mean egg weight and body weight of hens at the end of the laying period. As a result the egg weight of these hens (0 µg B_{12} supplemented per kg feed) was 2 % less, and the body weight was 4 % less and the feed conversion was increased by 3 % compared to hens with 5 µg B_{12} supplemented to the diet.

Table 6:
Egg composition (n = 163 to 181/ eggs/group, from three collection periods) (Means, SD, P-value)

Treatments		Egg weight g/egg	Yolk %	Albumen %	Shell %	Yolk colour Roche fan
B_{12} µg/kg	Co mg/kg					
0	0	62.4 ± 4.6	27.8 ± 2.0	60.0 ± 2.4	12.2 ± 1.0	12.7 ± 0.6
5	0	63.3 ± 4.5	27.6 ± 1.9	60.3 ± 2.3	12.1 ± 0.9	12.6 ± 0.6
10	0	63.3 ± 6.4	28.1 ± 2.1	59.2 ± 2.5	12.6 ± 1.1	12.6 ± 0.6
20	0	62.6 ± 5.6	27.8 ± 2.3	60.0 ± 2.7	12.2 ± 0.9	12.8 ± 0.6
0	0.6	62.4 ± 4.2	28.2 ± 2.0	59.3 ± 2.4	12.4 ± 1.2	12.8 ± 0.5
5	0.6	63.3 ± 5.9	28.0 ± 2.4	59.5 ± 2.8	12.4 ± 0.9	12.7 ± 0.6
10	0.6	64.7 ± 5.2	27.9 ± 2.5	59.9 ± 2.8	12.2 ± 1.0	13.0 ± 0.6
20	0.6	63.4 ± 4.6	27.6 ± 2.4	60.1 ± 2.8	12.2 ± 1.1	12.5 ± 0.8
ANOVA, P-value						
B_{12}		0.001	0.24	0.05	0.048	0.002
Co		0.048	0.38	0.24	0.28	0.26
B_{12} x Co		0.21	0.06	0.001	0.001	0.001

Table 7:
Organ mass, breast meat mass (g/hen) and hematocrit (%) in blood at the end of the trial (n = 8) (Means, SD, P-value)

Treatments		Heart	Liver	Spleen	Breast meat	Hematocrit
B_{12} µg/kg	Co mg/kg					
0	0	6.8 ± 0.7	34.4 ± 4.9	1.3 ± 0.4	132.0 ± 24.5	25.5 ± 3.3
20	0	6.8 ± 0.9	28.8 ± 6.8	1.4 ± 0.2	144.2 ± 22.5	28.1 ± 1.9
0	0.6	6.8 ± 1.0	39.0 ± 8.4	1.8 ± 0.4	140.6± 22.2	26.6 ± 2.3
20	0.6	7.2 ± 0.8	32.7 ± 3.2	1.8 ± 0.4	140.6 ± 19.3	28.0 ± 2.3
ANOVA, P-value						
B_{12}		0.55	0.01	0.71	0.44	0.03
Co		0.66	0.06	0.004	0.75	0.57
B_{12} x Co		0.45	0.89	0.46	0.44	0.53

Our laying hens originated from a parent flock with feeding according to the nutritional requirements of breeder hens and were also fed throughout the rearing period of chickens in accordance with nutrient requirements. Therefore it can be assumed that the storage of vitamin B_{12} into the hen body had an optimum B_{12} depot at the start of the laying period.

Scott et al. (1982) observed that two to five months would be needed to deplete hens of vitamin B_{12} stores to such an extent that progeny would hatch with low vitamin B_{12} reserves. On the question of vitamin B_{12} storage in the egg, Robel (1983) established that changes in vitamin levels (also in vitamin B_{12}) deposited in the eggs are related to the aging process of the turkey breeder hen. Souires and Naber (1992) concluded from the results of a study of vitamin profiles of eggs – which concentrations of vitamin B_{12} between 1.3 and 2.9 µg/100 g egg yolks are found, and for this reason, 8 µg vitamin B_{12} per kg diet appeared to be needed to support maximum hatchability and egg weight.

In the present study a vitamin B_{12} content of 8 µg per kg was analyzed in the complete control diet, this value resulted from microbiological contaminations of feed stuffs. But this content was not adequate for the laying hens of the Control to produce eggs with the same egg weight as compared to B_{12}-supplemented groups. A supplementation of 5 µg vitamin B_{12} per kg diet contributed to significantly higher egg weight, daily egg mass production and improved feed conversion. The results do not allow any clear conclusion, if this result is the outcome of the B_{12} content from microbiological contamination plus the supplemented 5 µg vitamin B_{12} per kg diet or this is exclusively the result from the supplemented 5 µg vitamin B_{12} per kg diet.

In studies with laying hens Bunchasak and Kachana (2009) measured no effect of B_{12} supplementation on feed intake or laying intensity, this is comparable with the own results. From a series of hen studies, Keshavarz (2003) concluded that certain manipulations of the combination of methionine, choline, folic acid, and vitamin B_{12} have the potential to reduce egg weight and improve shell quality without affecting egg production during the latter stages of the laying period.

In this study the supplementation of B_{12} significantly increased the body weight of the laying hens at the end of the trial. Studies on broilers showed that a deficiency of B_{12} in growing chickens results in reduced weight gain and feed intake (Halle et al., 2011), along with poor feathering and nervous disorders (Leeson and Summers, 2001). While a deficiency may lead to perosis, this is probably a secondary effect due to a dietary deficiency of methionine, choline or betaine as sources of methyl groups. Vitamin B_{12} may alleviate the perosis condition due to its effect on the synthesis of methyl groups. Further clinical signs reported in poultry are anaemia, gizzard erosion, and fatty infiltration of heart, liver and kidneys. A deficiency of Vitamin B_{12} in laying hens also resulted in a significantly higher liver mass. The measured haematocrit in the B_{12} supplemented hen groups was higher and was in agreement with the literature (Mehner and Hartfiel, 1983, 21 to 55 %).

The supplementation of Co alone without vitamin B_{12} in the diets in the present study did not change daily feed intake or laying intensity but significantly improved egg weight. The body weight of the hens at the end of the trial was not changed in the group with Co supplementation compared to Control. In studies with pigs it was observed that vitamin B_{12} – deficiency signs, including an accumulation of serum homocysteine, can be attenuated by Nickel and Co, although the mode of action seems to differ (Stangl et al., 2000). Previous studies have established that Co ions induce a series of metabolic changes in experimental animals. Co supplementation only improved homocysteine accretion in serum, whereas the vitamin B_{12} status remained completely unchanged (Goncharevskaia et al., 1985; Rosenberg and Kappas, 1989; Zhang et al., 1998). These findings indicate that there is no improvement in the vitamin B_{12} status and protein synthesis of the meat after supplementation of Co.

Furthermore it cannot be excluded that hens in the present study obtained a part of their vitamin B_{12} requirement more indirectly by ingesting faeces in the litter. The faeces bacterial flora is an important source of vitamins for coprophagic animals. The supplementation of Co to laying hen diets supported the vitamin B_{12} synthesis of bacteria in the digestive tract.

The combined supplementation of vitamin B_{12} and Co in the laying hen diets did not provide recognizable advantages relating to feed intake or laying performance.

It can be concluded from these observations that a supplementation of 5 µg vitamin B_{12} per kg laying hen feed was sufficient to compensate the deficiencies on feed intake and laying performance. The results allow the conclusion that an additional supplementation of Co to feed does not have additional advantages for the birds.

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