Effects of Dietary Cobalt Supplementation Levels on Digestion and Metabolism of Rex Rabbits from Weaning to 3 Months of Age (Postprint)

Authors: Gao Qin, Liu Lei, Liu Gongyan, Sun Haitao, Li Fuchang

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Abstract

This experiment aimed to investigate the effects of dietary cobalt supplementation levels on apparent digestibility of dietary nutrients, nitrogen metabolism, pancreatic digestive enzyme activities, serum biochemical indices, and cobalt metabolism in weaned Rex rabbits from weaning to 3 months of age. Two hundred weaned Rex rabbits with similar body weight were selected and randomly divided into 5 groups with 40 replicates per group and 1 rabbit per replicate. The five groups were fed experimental diets supplemented with 0 (control), 0.1, 0.4, 1.6, and 6.4 mg/kg cobalt (in the form of cobalt sulfate) in the basal diet, respectively, and a 53-day feeding trial was conducted after a 7-day pre-feeding period. The results showed that dietary cobalt supplementation level had no significant effect on the apparent digestibility of various nutrients in the diet ($P>0.05$). However, the apparent digestibility of dietary DM, CP, and cobalt all exhibited a trend of first increasing and then decreasing with increasing dietary cobalt supplementation level; the apparent digestibility of DM and CP was highest at the cobalt supplementation level of 1.6 mg/kg, while the apparent digestibility of cobalt was highest at 0.4 mg/kg. Dietary cobalt supplementation level had no significant effects on fecal nitrogen (FN), digestible nitrogen (DN), nitrogen apparent digestibility (NAD), or nitrogen biological value (NBV) ($P>0.05$), but had significant effects on nitrogen intake (IN), urinary nitrogen (UN), nitrogen retention (RN), and nitrogen utilization rate (NUR) ($P<0.05$). With increasing dietary cobalt supplementation level, IN, RN, and NUR all first decreased, then increased, and then decreased again, with the highest values observed at the cobalt supplementation level of 1.6 mg/kg, while UN decreased gradually. Dietary cobalt supplementation level had a significant effect on pancreatic trypsin activity ($P<0.05$), which was highest at the cobalt supplementation level of 1.6 mg/kg. Dietary cobalt supplementation level had a significant effect on serum urea content ($P<0.05$); with increasing dietary cobalt supplementation level, serum urea content first decreased and then increased, with the lowest value

observed at the cobalt supplementation level of 1.6 mg/kg. Dietary cobalt supplementation level had no significant effect on serum total protein and albumin contents (P>0.05). Dietary cobalt supplementation level had significant effects on cobalt contents in feces, urine, muscle, spleen, and kidney (P<0.05), and the cobalt contents in feces, urine, muscle, spleen, and kidney all increased continuously with increasing dietary cobalt supplementation level. Based on the measured indices in this experiment, the appropriate dietary cobalt supplementation level for weaned Rex rabbits from weaning to 3 months of age was 0.4–1.6 mg/kg (with measured dietary cobalt content of 0.60–1.83 mg/kg).

Full Text

Effects of Dietary Cobalt Supplemental Level on Digestion and Metabolism of Weaned to 3-Month-Old Rex Rabbits

GAO Qin[1], LIU Lei[1]*, LIU Gongyan[1], SUN Haitao[2], LI Fuchang[1]
(1. College of Animal Science and Technology, Shandong Agricultural University, Tai' an 271018, China;
2. Animal Husbandry and Veterinary Institute, Shandong Academy of Agricultural Sciences, Jinan 250100, China)

Abstract: This experiment was conducted to investigate the effects of dietary cobalt supplementation on nutrient apparent digestibility, nitrogen metabolism, pancreatic digestive enzyme activities, serum biochemical indices, and cobalt metabolism in weaned Rex rabbits from 35 days to 3 months of age. Two hundred weaned Rex rabbits with similar body weight were randomly allocated into five groups with 40 replicates per group (one rabbit per replicate). The five groups were fed experimental diets supplemented with 0 (control), 0.1, 0.4, 1.6, or 6.4 mg/kg cobalt (as cobalt sulfate) added to a basal diet. Following a 7-day adaptation period, a 53-day feeding trial was conducted. The results showed that dietary cobalt supplementation had no significant effect on the apparent digestibility of any nutrient (P>0.05). However, the apparent digestibility of dry matter (DM), crude protein (CP), and cobalt tended to increase initially and then decrease with increasing cobalt levels, with DM and CP digestibility peaking at 1.6 mg/kg cobalt supplementation and cobalt digestibility peaking at 0.4 mg/kg. Cobalt supplementation had no significant effects on fecal nitrogen (FN), digestible nitrogen (DN), nitrogen apparent digestibility (NAD), or nitrogen biological value (NBV) (P>0.05), but significantly affected nitrogen intake (IN), urinary nitrogen (UN), retention nitrogen (RN), and nitrogen utilization rate (NUR) (P<0.05). As dietary cobalt increased, IN, RN, and NUR initially decreased, then increased, then decreased again, all reaching their maximum values at 1.6 mg/kg cobalt, while UN decreased gradually. Pancreatic trypsin activity was significantly affected by cobalt level (P<0.05) and was highest at 1.6 mg/kg cobalt. Serum urea content was also significantly affected (P<0.05), decreasing initially then increasing with cobalt supplementation, reaching its lowest value at 1.6 mg/kg. No significant effects were observed on serum total

protein or albumin contents (P>0.05). Cobalt content in feces, urine, muscle, spleen, and kidney was significantly affected by supplementation (P<0.05) and increased continuously with higher cobalt levels. Based on these comprehensive results, the appropriate dietary cobalt supplemental level for weaned to 3-month-old Rex rabbits is 0.4–1.6 mg/kg (corresponding to measured dietary cobalt concentrations of 0.60–1.83 mg/kg).

Keywords: cobalt; Rex rabbits; nutrient apparent digestibility; nitrogen metabolism; cobalt metabolism

Introduction

In 1935, Underwood and Marston independently discovered that sheep wasting disease was caused by cobalt deficiency, establishing cobalt as an essential trace element for animals—the sixth such element to be confirmed. Cobalt is a critical component of vitamin B12 (cobalamin) and participates in hematopoiesis and nutrient digestion and metabolism through vitamin B12, thereby influencing protein, fat, and carbohydrate metabolism. During metabolic processes, cobalt can activate phosphatases, arginase, and catalase while inhibiting cytochrome oxidase and succinate dehydrogenase activities, improving glucose and nitrogen absorption in basal metabolism and promoting animal growth. In rabbits, our research group previously found that dietary cobalt supplementation at various levels (0.1–6.4 mg/kg) had no significant effect on daily weight gain or feed conversion efficiency in growing Rex rabbits. The NRC (2005) reported that sulfur-containing amino acids, particularly cysteine, readily form complexes with cobalt, reducing its absorption. Kincaid et al. demonstrated that serum vitamin B12 concentrations decline in dairy cows during the dry period (especially 55–20 days prepartum), and dietary cobalt supplementation can increase vitamin B12 synthesis in the rumen while elevating vitamin B12 levels in colostrum and milk. Schwarz et al. confirmed that growing and mature cattle require more cobalt than NRC recommendations. These findings indicate substantial species differences in cobalt requirements.

Cobalt also promotes fiber-decomposing bacterial activity, thereby enhancing fiber digestion. Cobalt deficiency leads to pica, anorexia, poor growth, emaciation, anemia, decreased blood vitamin B12, elevated methylmalonic acid and homocysteine, reduced red blood cells, and decreased hemoglobin. Recent years have seen increased attention from nutritionists due to cobalt' s important biological functions. Dietary cobalt can improve feed digestibility, particularly for poor-quality feeds. Krasnodebska reported that supplementing wethers fed semi-synthetic diets with 1 mg cobalt daily increased crude fiber digestibility by 13.4%. Singh et al. found that supplementing cobalt to calves fed low-quality forage with urea significantly improved the utilization of non-protein nitrogen and other nutrients. Mu Xiumei et al. showed that dietary cobalt and vitamin B12 supplementation benefited growth and increased daily weight gain in weaned lambs. Hertz et al. reported that dietary cobalt improved survival rate, fertilization hatching rate, and protein synthesis in carp. However, most cobalt

research has focused on ruminants and aquatic animals, and whether cobalt supplementation affects nutrient digestion and metabolism in rabbits remains unknown. Therefore, this experiment supplemented different cobalt levels in rabbit diets and measured nutrient apparent digestibility, nitrogen metabolism, and cobalt metabolism to further determine appropriate dietary cobalt levels and provide reference values for cobalt requirements in growing Rex rabbits.

1. Materials and Methods

1.1 Experimental Period and Location

The feeding trial was conducted from October 1 to December 1, 2015, at the animal experimental facility of the College of Animal Science and Technology, Shandong Agricultural University. The rabbit house was a closed single-story building with cement floors, and rabbits were housed individually in cages. Sample analyses were completed at the Animal Husbandry Platform Laboratory of the same college.

1.2 Experimental Animals and Management

Two hundred 35-day-old weaned Rex rabbits with an average body weight of (855.6±22.0) g (half male and half female) were randomly divided into five groups by sex and weight, with 40 replicates per group and one rabbit per replicate. Initial body weight did not differ significantly among groups ($P>0.05$). The five groups were fed experimental diets supplemented with 0 (control), 0.1, 0.4, 1.6, or 6.4 mg/kg cobalt, with measured cobalt concentrations in the pelleted diets of 0.27, 0.35, 0.60, 1.83, and 6.62 mg/kg, respectively. The basal diet was formulated according to De Blas et al.' s standards for growing rabbits, with composition and nutrient levels shown in Table 1 . Cobalt was supplemented as cobalt sulfate (purchased from Guangxi Nanning Yiwei Feed Technology Co., Ltd., cobalt content (1.00±0.05)%, batch number 20150308-5). The trial consisted of a 7-day adaptation period followed by a 53-day experimental period. Rabbits were fed twice daily (morning and evening). The rabbit house, cages, feed boxes, and watering equipment were thoroughly cleaned and disinfected before the trial. During the experiment, rabbits had free access to feed and water under natural lighting and ventilation, with rabbit house disinfection conducted every 3–5 days.

Table 1 Basal diet composition and nutrient levels (air-dry basis)

Ingredients	Content
Corn	
Wheat middling	
Corn germ meal	
Sunflower meal	
Rice hull	

Ingredients	Content
Guinea grass	
Soybean meal	
Expanded soybean meal	
Soybean oil	
Soybean phosphatides	
Premix[1]	
Lysine	
Methionine	
Total	

[1]The premix provided the following per kg of diet: VA 12,000 IU, VD 900 IU, VE 50 mg, VK 1.5 mg, VB 1.5 mg, VB 5 mg, VB 40 mg, VB 50 mg, VB 0.5 mg, VB 2.5 mg, VB 0.02 mg, choline 600 mg, biotin 0.2 mg, K 7 mg, Mg 3 mg, Fe 60 mg, Zn 60 mg, Cu 40 mg, Mn 9 mg, I 1 mg, Se 0.2 mg, limestone 15,000 mg, NaCl 5,000 mg, Lys 1,000 mg, Met 2,000 mg, 10% bacitracin zinc 300 mg.

[2]Digestible energy (DE) was a calculated value, while other nutrient levels were measured values.

1.3 Sample Collection and Preparation

Six days before the end of the feeding trial, eight rabbits were randomly selected from each group and transferred to disinfected metabolic cages for individual housing, where they were fed the corresponding experimental diets with free access to feed and water. Following a 3-day adaptation period, fecal and urine samples were collected continuously for three days from each rabbit and stored at 4°C in sealed containers, while daily feed intake, fecal output, and urine volume were recorded. Fresh feces were weighed, and a portion was mixed with 10% sulfuric acid for nitrogen fixation at a consistent daily sampling ratio, then oven-dried at 65°C for 72 hours to obtain air-dry weight. The three-day air-dried fecal samples were then pooled, ground, mixed, and stored at -20°C for analysis. Urine volume was measured, and a proportional aliquot was collected in 250 mL plastic bottles with 5 mL concentrated sulfuric acid for nitrogen fixation at a consistent daily sampling ratio, mixed, and stored at -20°C.

On day 54 of the trial, eight rabbits from each group were weighed after overnight fasting, and 10 mL blood samples were collected via cardiac puncture. Serum was separated and stored at -20°C. Following blood collection, rabbits were slaughtered and tissue samples were collected and stored in liquid nitrogen for subsequent analysis.

1.4 Measurements

1.4.1 Dietary Nutrient Apparent Digestibility Energy was determined using a Parr-6200 oxygen bomb calorimeter. Dry matter (DM) content was measured according to GB/T 6435–2006. Crude fiber (CF) was determined by acid-alkali washing method. Ether extract (EE) was measured by Soxhlet extraction. Crude ash content was determined by high-temperature incineration. Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were measured using the Van Soest fiber washing method. Calcium (Ca) was determined by potassium permanganate titration, phosphorus (P) by molybdenum yellow colorimetry, and cobalt content by the method described in GB/T 13884–2003. Detailed procedures for all measurements can be found in "Feed Analysis and Feed Quality Detection Technology" edited by Zhang Liying. Dietary nutrient apparent digestibility was calculated using the following formula:

Apparent digestibility of dietary nutrients (%) = 100 × (amount of nutrient intake - amount of corresponding nutrient in feces) / amount of nutrient intake

1.4.2 Nitrogen Metabolism Indices Nitrogen content in diets, feces, and urine was determined by the Kjeldahl method as described in "Feed Analysis and Feed Quality Detection Technology" edited by Zhang Liying. Nitrogen metabolism indices were calculated as follows:

- Digestible nitrogen (DN, g/d) = Nitrogen intake (IN) - Fecal nitrogen (FN)
- Retention nitrogen (RN, g/d) = IN - FN - Urinary nitrogen (UN)
- Nitrogen apparent digestibility (NAD, %) = 100 × DN / IN
- Nitrogen utilization rate (NUR, %) = 100 × RN / IN
- Nitrogen biological value (NBV, %) = 100 × RN / DN

1.4.3 Pancreatic Digestive Enzyme Activities Pancreatic trypsin and chymotrypsin activities were measured using commercial assay kits from Nanjing Jiancheng Bioengineering Institute. The principle for trypsin: trypsin catalyzes the hydrolysis of arginine ethyl ester substrate, increasing absorbance at 253 nm, with enzyme activity calculated from absorbance changes. For chymotrypsin: chymotrypsin hydrolyzes casein to produce phenolic amino acids, which reduce phenol reagent to a blue compound measurable by colorimetry.

1.4.4 Serum Biochemical Indices Serum total protein (TP), albumin (ALB), and urea (UR) contents were measured using kits from Wako Pure Chemical Industries, Ltd., Japan, on a Hitachi 7020 automatic analyzer according to kit instructions.

1.4.5 Cobalt Content in Diet, Feces, Urine, and Tissues Diet, feces, muscle, spleen, and kidney samples were dried at 105°C, ashed in a muffle fur-

nace until carbon-free, digested with HNO -HClO (3:1), boiled for several minutes, cooled, diluted, and filtered. Cobalt content was determined by atomic absorption spectrophotometry. Serum and urine were digested with HNO -HClO (4:1) before cobalt determination by atomic absorption spectrophotometry.

1.5 Data Processing and Analysis

Data were analyzed using the GLM procedure of SAS 9.1.3 software, with Duncan' s multiple range test used for post-hoc comparisons.

2. Results

2.1 Effects of Dietary Cobalt Level on Nutrient Apparent Digestibility

As shown in Table 2 , dietary cobalt supplementation had no significant effect on the apparent digestibility of DM, CP, or cobalt (P>0.05). However, the apparent digestibility of DM, CP, and cobalt tended to increase initially then decrease with higher cobalt levels, with DM and CP digestibility peaking at 1.6 mg/kg cobalt and cobalt digestibility peaking at 0.4 mg/kg. No significant effects were observed on the apparent digestibility of energy, EE, ash, CF, NDF, ADF, ADL, Ca, or P (P>0.05).

Table 2 Effects of dietary cobalt supplemental level on nutrient apparent digestibility of weaned to 3-month-old Rex rabbits (n=8)

Item	Dietary cobalt supplemental level (mg/kg)	R-MSE	P-value
Dry matter (DM)			
Energy			
Crude protein (CP)			
Ether extract (EE)			
Ash			
Crude fiber (CF)			
Neutral detergent fiber (NDF)			
Acid detergent fiber (ADF)			
Lignin (ADL)			
Calcium (Ca)			
Cobalt (Co)			

In the same row, values with the same letter superscripts mean no significant difference (P>0.05), while different lowercase letters indicate significant difference (P<0.05), and different capital letters indicate highly significant difference (P<0.01). The same as below.

2.2 Effects of Dietary Cobalt Level on Nitrogen Metabolism

As shown in Table 3 , dietary cobalt level significantly affected IN (P<0.05), with the 0.1 mg/kg group showing significantly lower IN than the control group (P<0.05). Cobalt supplementation significantly affected UN, RN, and NUR (P<0.05). As cobalt levels increased, RN and NUR initially decreased, then increased, then decreased again, both reaching maximum values at 1.6 mg/kg cobalt, while UN decreased gradually. No significant effects were observed on FN, DN, NAD, or NBV (P>0.05).

Table 3 Effects of dietary cobalt supplemental level on nitrogen metabolism of weaned to 3-month-old Rex rabbits (n=8)

Item	Dietary cobalt supplemental levels (mg/kg)	R-MSE	P-value
IN (g/d)	5.48 , 5.31 , 5.42 , 5.50 , 5.40		
FN (g/d)	1.40		
UN (g/d)	1.28 , 1.08 , 1.07 , 1.04 , 1.04		
DN (g/d)			
RN (g/d)	2.64 , 2.54 , 2.57 , 2.99 , 2.96		
NAD (%)			
NUR (%)	48.29 , 47.67 , 47.38 , 54.70 , 54.40		
NBV (%)			

2.3 Effects of Dietary Cobalt Level on Pancreatic Digestive Enzyme Activities

As shown in Table 4 , dietary cobalt level significantly affected pancreatic trypsin activity (P<0.05) but had no significant effect on chymotrypsin activity (P>0.05). Trypsin activity initially increased then decreased with higher cobalt levels, reaching its maximum at 1.6 mg/kg cobalt.

Table 4 Effects of dietary cobalt supplemental level on digestive enzyme activities in pancreas of weaned to 3-month-old Rex rabbits (n=8)

Item	Dietary cobalt supplemental level (mg/kg)	R-MSE	P-value
Trypsin (U/mg)	8.86 , 10.10 , 14.07 , 14.09 , 10.34		
Chymotrypsin (U/mg)			

2.4 Effects of Dietary Cobalt Level on Serum Biochemical Indices

As shown in Table 5 , dietary cobalt level significantly affected serum UR content (P<0.05), which initially decreased then increased with higher cobalt levels, reaching its minimum at 1.6 mg/kg cobalt. No significant effects were observed on serum TP or ALB contents (P>0.05).

Table 5 Effects of dietary cobalt supplemental level on serum biochemical indices of weaned to 3-month-old Rex rabbits (n=8)

Item	Dietary cobalt supplemental levels (mg/kg)	R-MSE	P-value
TP (mmol/L)			
ALB (mmol/L)			
UR (mmol/L)	7.59 , 5.18 , 5.15 , 4.02 , 6.12		

2.5 Effects of Dietary Cobalt Level on Cobalt Metabolism

As shown in Table 6 , dietary cobalt level significantly affected cobalt content in feces, urine, muscle, spleen, and kidney (P<0.05), with cobalt content in these tissues increasing continuously as dietary cobalt levels rose. No significant effect was observed on serum cobalt content (P>0.05).

Table 6 Effects of dietary cobalt supplemental level on cobalt metabolism of weaned to 3-month-old Rex rabbits (n=8)

Item	Dietary cobalt supplemental level (mg/kg)	R-MSE	P-value
Fecal (ng/g)	0.30 , 0.47 , 0.85 , 2.06 , 8.26	<0.0001	
Urine (ng/mL)	0.05 , 0.10 , 0.21 , 0.35 , 1.27	<0.0001	
Serum (ng/mL)			
Muscle (ng/g)	1.51 , 1.52 , 1.90 , 2.09 , 3.29		
Spleen (ng/g)	4.42 , 5.08 , 5.23 , 6.23 , 7.27		
Kidney (ng/g)	2.47 , 3.27 , 4.01 , 4.36 , 5.89		

3. Discussion

3.1 Effects of Dietary Cobalt on Nutrient Apparent Digestibility

Rex rabbits require cobalt for vitamin B12 synthesis, and vitamin B12 acts as a coenzyme for methylmalonyl-CoA mutase and 5-methyltetrahydrofolate methyltransferase, participating in gluconeogenesis and methionine synthesis. In sheep, Becker et al. found that cobalt-deficient sheep had significantly higher apparent digestibility of CF than cobalt-sufficient sheep, while Pal et al. reported that feeding 0.25 mg/kg body weight of cobalt sulfate significantly improved digestibility of total carbohydrates, nitrogen-free extracts, and CF. Kadim et al. compared goats fed low-cobalt diets with those receiving subcutaneous cobalt injections (2,000 g) and found that low dietary cobalt reduced apparent digestibility of DM, CP, and EE as well as digestible energy. Lopez-Guisa et al. noted that providing cobalt above NRC recommendations (0.24 mg/kg) to young cattle fed low-quality diets improved digestibility. Tomlinson et al. reported that cobalt' s nutritional function extends beyond ruminal vitamin B12 synthesis to enhancing fiber-decomposing bacterial activity and promoting fiber

digestion. However, Saxena et al. found that supplementing calf diets with 0.25, 0.30, or 0.40 mg/kg cobalt had no significant effect on apparent digestibility of DM, CP, EE, or CF. Hussein et al. observed no significant effect of high-dose cobalt supplementation (5–30 mg/kg) on DM or CF digestibility in vitro, and Kišidayová et al. similarly found that supplementing cobalt (2, 4, or 8 mg/kg) to a basal diet containing 0.2 mg/kg cobalt did not promote degradation of DM, NDF, or ADF in vitro. The current study found no significant effect of dietary cobalt on nutrient apparent digestibility in Rex rabbits, though DM, CP, and cobalt digestibility tended to increase then decrease with higher cobalt levels. These inconsistent results across studies suggest species differences in how cobalt affects nutrient digestibility.

3.2 Effects of Dietary Cobalt on Nitrogen Metabolism

As an essential component of vitamin B12, cobalt is closely related to nitrogen assimilation and synthesis of heme and muscle protein. Cobalt deficiency slows vitamin B12 synthesis, impairing utilization of dietary nitrogenous compounds. Cobalt promotes urea utilization and cellulose digestion in cattle fed non-legume hay and increases growth rate. Mburu et al. fed East African goats a cobalt-deficient diet and found higher UN compared to goats receiving adequate cobalt (as cobalt sulfate), indicating reduced dietary nitrogen fermentation in the rumen and increased nitrogen loss, leading to decreased growth and poor body condition. Roginski et al. reported that dietary cobalt improved growth rate, survival, fertilization hatching rate, and protein synthesis in carp. Anadu et al. found that adding cobalt chloride to tilapia diets accelerated protein synthesis. In the current study, cobalt supplementation had no significant effect on FN, DN, NAD, or NBV in Rex rabbits but significantly affected IN, UN, RN, and NUR. As cobalt levels increased, IN, RN, and NUR initially decreased, then increased, then decreased again, while UN decreased gradually, with IN, RN, and NUR reaching maximum values at 1.6 mg/kg cobalt. These results suggest that appropriate cobalt supplementation can improve protein digestibility and utilization in Rex rabbits, possibly due to increased activity of protein-digesting enzymes in the pancreas, while decreased serum UR content indirectly confirms improved protein utilization. Serum TP and ALB contents did not change significantly with cobalt supplementation, which differs from Li Qingyun et al.' s findings that 1.0 and 2.0 mg/kg cobalt supplementation significantly increased plasma TP and ALB in Peking ducks, likely due to species differences.

3.3 Effects of Dietary Cobalt on Cobalt Metabolism

Tissue cobalt content serves as a valuable indicator of cobalt nutritional status because sample contamination is reduced, improving measurement accuracy. The primary site of cobalt absorption is the posterior small intestine, which partially shares intestinal mucosal transport pathways with iron. Animal body cobalt content is low (0.03–0.06 mg/kg), with 43% distributed in muscle, 14% in bone, and the remaining 43% in various soft tissues. Cobalt concentrations are

relatively high in liver and kidney, followed by adrenal gland, spleen, pancreas, and bone, with lower levels in other tissues and organs. Blood cobalt content is very low and highly variable, with whole blood cobalt at 38 g/dL and plasma cobalt at 0.5–0.7 mg/dL, indicating that most cobalt resides in red blood cells. Most plasma cobalt forms unstable compounds with - and -globulins, with a small portion existing as free ions, making blood cobalt content reflective of dietary cobalt status. In this study, serum cobalt content did not change significantly with dietary cobalt level. Previous research indicates that serum element concentrations remain relatively stable and are generally unaffected by supplementation levels, whereas the liver, as the primary metabolic organ for elements, is significantly affected by dietary element content and typically shows positive correlation. In this study, muscle, liver, and kidney cobalt contents in Rex rabbits increased continuously with higher dietary cobalt levels. Cobalt is primarily excreted as cations in urine, with smaller amounts in bile, while most unabsorbed cobalt is excreted in feces. The significantly higher fecal and urinary cobalt content in the 6.4 mg/kg supplementation group likely reflects cobalt excess in the diet.

Based on comprehensive evaluation of all measured indices, the appropriate dietary cobalt supplemental level for weaned to 3-month-old Rex rabbits is 0.4–1.6 mg/kg (corresponding to measured dietary cobalt concentrations of 0.60–1.83 mg/kg).

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Note: Figure translations are in progress. See original paper for figures.