Reviews on the mineral provision in ruminants (X): COBALT METABOLISM AND REQUIREMENTS IN RUMINANTS

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PREFACE

In the Netherlands the ´Handleiding Mineralenonderzoek bij rundvee in de praktijk´[1] is a well-known publication that has been used already for decades as a guide to trace and treat mineral disorders in cattle. The fifth edition of this guidebook was published in 1996. The content of this publication was largely identical to that of the fourth edition (1990). Therefore the (independent) committee that is responsible for the contents of the guidebook (the 'Commissie Onderzoek Minerale Voeding'[2], COMV) decided in 2000 that a thorough revision was desired.
The committee was of the opinion that, if possible, the available scientific literature should be summarized and evaluated once again. Furthermore, attention should be paid to the mineral provision of categories of cattle other than dairy cattle, as well as to that of sheep and goats. Finally, the basic principles for the calculation of the mineral requirements should be described in a transparent way.

The intended revision was made possible as the Dutch 'Ministerie van Landbouw, Natuur en Voedselkwaliteit' (LNV), the 'Productschap Diervoeder' and the 'Productschap Zuivel'[3] were willing to subsidize this extensive and ambitious project.
The COMV decided to execute the project as follows.

- External experts, invited by the COMV, should summarize and evaluate the relevant literature in a so-called 'basal document' (with two exceptions to be written in English).
- Subsequently, these documents should be critically evaluated by the COMV.
- These basal documents should then be used to write and arrange the several chapters of the revised 'Handleiding'.

The revised 'Handleiding' is available (in the Dutch language) since October 2005, under the title 'Handleiding mineralenvoorziening rundvee, schapen en geiten.'[4] This book is published by the 'Centraal Veevoederbureau' (CVB; Central Bureau for Livestock Feeding) in Lelystad, as was also the case for the previous edition.

The COMV was of the opinion that the valuable basal documents, that became available during the course of this project, should be published too. By doing so everyone has the possibility to trace the basis for the text of the revised 'Handleiding'. The CVB was gladly willing to issue these documents as CVB Documentation reports. In connection with this the authors and the members of the COMV have disclaimed all rights and have assigned them to the Productschap Diervoeder, of which the CVB is one of the services.
For an overview of the CVB Documentation Reports that will appear in this context, you are referred to an Annex in the back of this report.
For the preparation of the present report on the Cobalt provision in ruminants the COMV expresses its gratitude to the author, dr. A.M. van den Top.

Utrecht/Lelystad, November 2005.

Professor dr. ir. A.C. Beynen	Dr. M.C. Blok
Chair of the COMV	Secretary of the COMV and Head of the CVB

The author, Dr. A.M. van den Top, expresses his thanks to the COMV, especially dr. M.C. Blok, prof. dr. A.Th. van 't Klooster and dr. J. Veling for critically reading the manuscript and their advice.

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1 Guidebook on mineral research for cattle in practice.
2 Committee for research on mineral nutrition
3 The Ministry for Agriculture, Nature and Food quality, the Product Board Animal Feed and the Dutch Dairy Board, respectively.
4 Guidebook mineral provision cattle, sheep and goats.

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CONTENT

LIST OF ABBREVIATIONS

Abbreviation	Unit	Description
BW		Body weight
DM		Dry matter
DMI	Kg	Dry matter intake
FIGLU		Formiminoglutamate
MMA		Methyl malonic acid
OWLD		Ovine white liver disease

1 FUNCTIONS OF COBALT IN THE BODY

The only known function of Co in ruminants is to promote vitamin B_{12} (cobalamin) synthesis in the rumen by anaerobic bacteria. For reviews on vitamin B_{12} formation and functions in the body, see references [35], [54] and [56].

The core of cobalamin consists of a corrin ring with a central Co atom. Depending on the valence state of Co, the cobalamin molecule is referred to as B_{12a}, hydroxycobalamin (Co^{3+}), B_{12r} (Co^{2+}) or B_{12s} (Co^{+}). B_{12s} is converted into 5'deoxyadenosylcobalamin, which is the coenzyme of methylmalonyl CoA mutase. This enzyme is the key enzyme in gluconeogenesis, as it converts methylmalonyl CoA (from e.g. propionyl CoA) into succinyl CoA. Disturbances of the function of this enzyme due to vitamin B_{12} deficiency raise plasma and urine MMA concentrations. As ruminants largely depend on gluconeogenesis for their glucose supply, Co (as a component of vitamin B_{12}) is of vital importance for these animals [41; 56]. On the other hand, rumen micro-organisms depend on the same enzyme for the production of volatile fatty acids. Besides true vitamin B_{12}, a number of vitamin B_{12}-like compounds (vitamin B_{12} analogues: corrinoids; e.g. 2-methyladenylcoamide [35]) is formed by ruminal micro-organisms. These compounds have little or no vitamin B_{12} activity and cannot be used by ruminants [61]. Vitamin B_{12} production may be reduced when the metabolisable energy content of the diet is increased. However the results are conflicting. E.g. changing the hay/maize ratio from 100:0 to 40:60, while keeping the dietary Co concentration constant, reduced vitamin B_{12} production from 1195 to 603 µg/day in wethers [57]), while corrinoid synthesis only slightly declined [35; 57]. However, in other experiments this effect could not be demonstrated [29]. Increasing DMI resulted in increased vitamin B_{12} production at the expense of corrinoids [57].

Finally, methylcobalamin is needed as a coenzyme of both (tetrahydrofolate) methyl-transferase and homocysteine transmethylase. The former enzyme is indirectly involved in the conversion of histidine into glutamate [35; 56]. Failure to form glutamate in this way results in increased urinary excretion of FIGLU (= formiminoglutamate)[5]. The latter enzyme is involved in the synthesis of methionine [56]. This is possibly an explanation for the fact that cattle and goats are less susceptible to Co deficiency than are sheep, because of their high requirement of S-containing amino acids needed for wool growth [61].

Cyanocobalamin, the usual commercial form of cobalamin, is considered to be an artificial form of cobalamin resulting from the isolation procedure [56].

[5] The conversion of histidine into glutamate consists of 4 steps: histidine – urocanate – 4-imidazolone 5 propionate – N-formiminoglutamate – glutamate. For the last step, methylcobalamin (containing Co) is rate-limiting [56].

2 VITAMIN B_{12} AND COBALT DISTRIBUTION

Like other water-soluble vitamins, vitamin B_{12} is poorly stored in the body. Upon repletion of Co-deficient sheep (0.1-10 mg Co/day), hepatic vitamin B_{12} concentrations reached an earlier plateau than serum vitamin B_{12} concentrations, indicating for a limited storage capacity in the liver [61]. In wether lambs fed a ration containing 0.15 ppm Co, mean hepatic vitamin B_{12} concentration was 0.77 µg/g fresh tissue. After subcutaneous administration of ^{58}Co-cyanocobalamin (500 µg over 20 days, 13µCi), liver and rumen contained the highest doses of ^{58}Co [55]. Selected results are given in Table 1.

Table 1. Selected results of ^{58}Co distribution in a vitamin B_{12}-deficient sheep after subcutaneous injection of 13 µCi ^{58}Co-cyanocobalamin over 20 days [55]

Tissue	percentage of total dose[a]
Liver	16.5
Kidneys	5.3
Spleen	0.6
Lungs	3.1
Brain	0.3
Heart	0.4
Muscles, bone marrow, blood, gall bladder	-
Rumen	10.5
Reticulum	1.9
Omasum	3.2
Abomasum	1.2
Ileum	3.8

[a] total recovery not given

Placental transfer is not marked and hepatic vitamin B_{12} concentrations of the neonate are only half those found in the dam [61].
In wether lambs on a ration containing 0.15 ppm Co, Co was mainly stored (all concentrations in ppm (DM)) in the liver (0.23) and kidney (0.24) [22]. Muscle (0.05) and spleen (0.07) contained only low concentrations, whereas Co concentrations in heart were intermediate (0.11)[6].

[6] As tissue Co concentrations were analysed by flameless atomic absorption spectroscopy and vitamin B_{12} concentrations were determined solely in liver samples, similarity of distribution patterns of Co and vitamin B_{12} between the tissues could not be compared.

3 EFFICIENCY OF VITAMIN B_{12} PRODUCTION AND ABSORPTION

Efficiency of Co incorporation into vitamin B_{12} is dependent on the Co status of the animal. In deficient sheep, this efficiency is 13%, whereas this value is only 3% when Co status is adequate [55]. When Co supply to sheep was decreased from ± 1.0 to ± 0.02 ppm (DM), assessed daily vitamin B_{12} production decreased from ± 650 to 40 µg/day within 5 days [55]. In sheep fed 0.047, 0.41 or 0.83 mg Co[7]/day estimated vitamin B_{12} production was 37, 1006 and 1553, respectively [15].

Cobalamins are selectively bound by intrinsic factor, a product of the parietal cells in the abomasum. Subsequently the vitamin is absorbed in the ileum. Based on comparison of performance data between orally and parenterally administered vitamin B_{12} in Co-deficient sheep, vitamin B_{12} absorption from gastrointestinal tract is assessed to be only 3-5% [25; 32; 55; 61]. This value cannot be referred to as "true" absorption. Apparent absorption of exogenous dietary ^{57}Co-cyanocobalamin is reported to range from 8-38% [49]. Major increases in Co supply cause considerable increases in cobalamin synthesis. Typically, increasing the dietary Co concentration from 0.1 to 0.5 ppm in sheep causes a 20-fold increase in cobalamin synthesis. Although the balance between the several analogues changes at different Co intakes, data on the partitioning between analogues and "active" vitamin B_{12} at varying Co intake are equivocal [61].

Thus, increasing Co intake is associated with a reduced efficiency of vitamin B_{12} synthesis and increased production of inactive vitamin B_{12} analogues [57].

No data are available on any differences in true vitamin B_{12} absorption due to variations in dietary composition (e.g. Co concentration, carbohydrate content). No data are available on adaptations in mucosal uptake in pregnant or neonatal animals. The cobalamin-intrinsic factor complex enters the enterocyte by receptor-mediated endocytosis. After release from the serosal side of the enterocyte cobalamin is bound to transcobalamins, transport proteins delivering cobalamin to the tissues. The concentration of these transcobalamins increases during infection and inflammation, suggesting an increased demand for cobalamin [61]. The receptor-mediated endocytosis of vitamin B_{12} has recently been reviewed in detail [52].

3.1 Cobalt solubility and vitamin B_{12} production from different cobalt sources

As Co is only necessary for vitamin B_{12} synthesis in the rumen [61], data on cobalt absorption from the gastrointestinal tract are irrelevant for normal ruminant physiology. In this respect, the solubility of Co sources in ruminal fluid is an important feature. Among the Co salts, only several forms of Co chloride and Co sulphate are soluble in water, e.g. $CoCl_2$.($2H_2O$ or $6H_2O$), $CoCl_3$, $CoSO_4$(H_2O or $7H_2O$). On the other hand, Co hydroxide and Co oxides ($CoOH_2$, CoO, $Co_2O_3.3H_2O$, Co_3O_4) are insoluble in water [65].

3.1.1 Cattle and goats

3.1.1.1 Cobalt absorption

Data on Co absorption are scarce. Ely et al. [10] found Co sulphate, Co chloride and Co carbonate to be equally toxic to dairy calves.

3.1.1.2 Tissue vitamin B_{12} concentrations after administration of different Co sources

No data are available on differences in blood or tissue vitamin B_{12} concentrations after the administration of different Co sources to cattle or goats.

[7] As $CoCl_2.6H_2O$.

3.1.2 Sheep

3.1.2.1 Cobalt absorption[8]

In wethers, liver and kidney Co concentrations were significantly lower when Co oxide instead of either Co sulphate, Co carbonate or Co glucoheptonate was given for 20 days [22]. Based on these tissue Co concentrations, relative bioavailabilities (Co sulphate RG[9] set at 100%) were 12-24% (oxide, FG[1]) <6% (oxide, RG), 115-140% (carbonate, FG), 91-133% (carbonate, RG) and 80% (glucoheptonate). These findings agree with those of others [2], who found lower liver Co concentrations when 40 ppm Co from either CoO or Co_3O_4 instead of $CoCO_3$ or $CoSO_4.7H_2O$ was fed. Liver Co concentrations were 0.38-0.91 (Co oxides) vs. 2.96 ppm (DM)(Co sulphate). Data on liver Co of the carbonate-treated group were not given. In a review of Henry [16], based on liver Co concentrations in sheep, relative bioavailability of Co sources ($CoSO_4.7H_2O$ set at 100%) is assessed to be 98 ($CoCO_3$, RG), 122 ($CoCO_3$, FG), 13 (Co_3O_4, RG), 31 (Co_3O_4, FG) or 80% (Co glucoheptonate). Cobaltous nitrate and Co chloride are reported to be "effective" or "suitable" sources of Co for ruminants, but no data are given [16; 41].

3.1.2.2 Tissue vitamin B_{12} concentrations after administration of different Co sources

In an experiment with wethers, no significant differences in serum or liver vitamin B_{12} concentrations could be demonstrated between either Co sulphate, oxide or carbonate [22]. Similarly, in another experiment [3], no differences in growth, serum and liver vitamin B_{12} concentrations were observed between sheep fed 300 mg supplemental Co / month as Co sulphate or Co oxide (mainly Co_3O_4). In contrast, in an *in vitro* experiment with rumen fluid, based on vitamin B_{12} concentrations in the medium after one-week incubation, the relative bioavailabilities of $CoSO_4.7H_2O$ (set at 100%), $CoCO_3$, Co glucoheptonate and Co_3O_4 were 100%, 91%, 84% and 0% [23]. The difference between Co_3O_4 and the other sources was significant. As the in vitro experiment revealed differences between Co sources that were not observed in vivo, it is not clear as to what extent the in vitro results are applicable in living animals.

3.1.3 Discussion and conclusions

Although evidence is equivocal, Co oxides seem to be less suitable Co sources. Differences between Co sulphate, Co carbonate and Co glucoheptonate seem to be minor. The cheapest and most convenient of these three sources can be used. Due to lack of data the effectiveness of Co nitrate and Co chloride cannot be judged.

3.2 Interactions influencing vitamin B_{12} production from dietary cobalt

Although sheep liver Co[10] concentrations have been reported to decrease when the inorganic sulphate concentration of the ration increases and to increase when Mo concentrations are increased, no suitable data are available on this subject [16]. For soil minerals and pH influencing Co uptake by plants see 6.1.

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8 Only Co concentrations were determined; as to what extent this Co was incorporated in cobalamine is not clear.

9 RG = reagent grade; FG = feed grade

10 Only Co concentrations were determined; as to what extent this Co was incorporated in cobalamine is not clear.

3.2.1 Interactions of cobalt and potassium metabolism

3.2.1.1 Cattle

Although increasing the K-content of the ration of cattle is reported to increase faecal Co excretion [44], as yet no suitable data are available to judge this effect.

3.2.1.2 Sheep

In sheep, feeding a diet containing 43 g K/kg DM transiently decreased plasma vitamin B_{12} concentrations when compared with a diet containing 8 g K/kg DM [53]. On average, both diets contained 124 µg Co/kg DM. During the 13-week experiment, from weeks 2 to 6 mean plasma vitamin B_{12} concentrations in the high-K group were ± 1500 pmol/L, whereas those in the low-K group were ± 2000 pmol/L. No clinical vitamin B_{12} deficiency symptoms were observed in either group.

3.2.1.3 Goats

No data are available on the interaction of Co and K in goats.

3.2.1.4 Conclusions

The K-concentrations used in the experiments resemble those encountered under current Dutch circumstances [51], and results may, therefore, be applicable in practice. However, although increasing the K content of the ration of ruminants seems to be detrimental to vitamin B_{12} supply, insufficient data are available to judge or quantify this effect.

3.3 Reclycling

In a vitamin B_{12}-deficient sheep injected with 500 µg ^{58}Co-labeled cyanocobalamin (13 µCi) for 20 days, 0.5% of the dose was recovered from the rumen contents. Thus, recycling of Co or vitamin B_{12} to the rumen is minor. From the duodenum contents <0.1% of dose could be recovered, whereas for the ileum these values declined from 1.8 to 0.4%. The contents of the caecum and more distal parts of the intestine contained percentages of the dose declining from 0.4 to <0.1%. These data obviously indicate for vitamin B_{12} secretion into and resorption from the small intestine, whereas secretion before the duodenum and resorption behind the ileum were minor. However, as bile did not contain labelled Co, the vitamin B_{12} secretion into the duodenum must have been via other routes [55].

3.4 Excretion

3.4.1 Vitamin B_{12} excretion

Data on vitamin B_{12} and Co excretion in ruminants are limited. In Co-loaded lactating dairy cows (17.8 mg/day), increases in urinary vitamin B_{12} up to 12 ng/mL were reported [64]. In Co-deficient ewes (0.01-0.05 ppm Co (DM)) daily urinary vitamin B_{12} excretion ranged from 0-0.17 µg/day. Faecal vitamin B_{12} excretion was 5-44 µg/day. Supplementation of oral Co[11] (± 1 ppm (DM)) increased faecal vitamin B_{12} excretion (86-92 µg/day), but urinary vitamin B_{12} excretion was much less increased (0-0.76 µg/day).

When 50 µg of cyanocobalamin was given by injection, the main relative increase was in urinary vitamin B_{12} excretion (15.2-17.7 µg/day), whereas increases in faecal vitamin B_{12} excretion were less pronounced (57-66 µg/day). In one Co-deficient ewe given 500 µg cyanocobalamin/day faecal excretion of vitamin B_{12} was 120 µg/day, whereas urinary excretion was only 0.24 µg/day, indicating for poor absorption of vitamin B_{12} from the

[11] As chloride.

gastrointestinal tract [55]. However, in another experiment with sheep 2-32% of label was recovered from urine when ^{57}Co-cyanocobalamin was infused into the duodenum [49]. As to what extent vitamin B_{12} production in and absorption from the large intestine takes place in ruminants is not clear.
In lambs fed either 0, 50, 100 or 150 µg supplemental Co/day, faecal vitamin B_{12} excretion was 1.43, 2.27, 2.47 and 2.77 µg vitamin B_{12}/g faecal DM [20]. The difference between the supplemented groups and the non-supplemented group was significant. The basal ration contained 0.042 ppm Co.

3.4.2 Cobalt excretion

In an experiment with one Co-deficient ewe given a dose of 0.2 mCi ^{60}Co[12], >90% of labelled Co was recovered from the faeces, whereas urinary Co excretion only accounted for ± 2-3% of dose [55]. Similar values (84-98% faecal excretion of labeled Co within 5-14 days) have been reported by other researchers for sheep and cattle [54]. In beef cows fed a single dose of either 0, 1, 2 or 4 Co pellets (30-g pellets containing 30% cobaltic oxide), increasing the number of pellets increased faecal Co excretion [21]. Data on faecal excretion are given in Table 2 (see Chapter 4).

[12] As cyanocobalamin via a rumen fistula.

4 COBALT REQUIREMENTS

Due to lack of data (only some milk data on vitamin B_{12} concentrations are available), no factorial estimation of Co and / or vitamin B_{12} requirements in ruminants can be made.

4.1 Cattle

4.1.1 Dairy cattle

Colostrum is reported to contain ± 7.5 [64] or 16-50 µg vitamin B_{12}/kg [27]. Literature values of the vitamin B_{12} content of mature bovine milk show considerable variation (µg vitamin B_{12}/kg) 0.2-14.5 [27] (literature synthesis), 3-7 [64] or 7.0 [19][13]. Values <1.0 µg vitamin B_{12}/kg are considered to indicate for deficiency, whereas 2.0-4.0 µg vitamin B_{12}/kg are considered to indicate for adequate Co supply [45]. Vitamin B_{12} concentrations in milk are lowest at 3 weeks after parturition and subsequently incline to colostral values up to 34 weeks of lactation [64].
The vitamin B_{12} content of milk depends on the dietary Co supply. In beef cows fed a single dose of 0, 1, 2 or 4 Co pellets (30 g-pellets containing 30% cobaltic oxide; unsupplemented ration containing 0.02-0.15 ppm Co (DM), usually <0.05 ppm (DM)), after 75 weeks of treatment both hepatic and milk vitamin B_{12} levels were significantly increased in the Co-treated groups [21]. The vitamin B_{12} levels in liver and milk are given in Table 2.

Table 2. Selected vitamin B_{12} levels at 75 weeks of treatment from an experiment with beef cows fed 0, 1, 2 or 4 Co pellets [21]

Number of pellets	Vitamin B_{12}					Faecal Co
	Plasma	Liver (fresh weight)		Milk		
	pmol/L	nmol/kg	ppm [a]	pmol/kg	µg/kg [a]	ppm (DM)
0	16	70	0.09	117 [x]	0.16	0.08 [x]
1	36	192	0.26	368 [xy]	0.50	0.13 [xy]
2	59	427	0.58	740 [y]	1.00	0.22 [yz]
4	63	496	0.67	1199 [y]	1.62	0.29 [z]

[a] calculated values to facilitate comparison; [x,y,z] means within a column with different superscript are significantly different

4.1.2 Sheep

Sheep milk is reported to contain 6.4 µg vitamin B_{12}/L (4723 pmol/kg), whereas goat milk is reported to contain 0.6 µg vitamin B_{12}/L (443 pmol/kg) [14].

4.2 Conclusion

Factorial estimation of Co requirements with the help of the equation used for other (trace) elements is impossible and, therefore, only rough estimations of allowances can be made.

[13] In pmol/kg, these values are 148-10701, 2214-5166 and 5166.

5 ALLOWANCES

Recommended allowances hardly differ between ruminant species. Hardly any data are given as to classify these recommendations (“minimal”, “optimal growth” etc.).

5.1 Cattle

For dairy cattle, the dietary Co requirement was estimated to be 0.07 ppm (DM) for growth and health [61] to 0.11 ppm (DM) [41]. When high-concentrate / high starch rations are fed (e.g. high yielding dairy cows; no data given), 0.2 ppm (DM) is recommended [30]. Besides secretion into milk, this possibly originates from increases in endogenous vitamin B_{12} losses or vitamin B_{12} being needed to utilize body reserves of other nutrients. For beef cattle, 0.10 ppm (DM) is recommended [40]. Values of 0.04-0.06 ppm Co (DM) should be regarded marginal [61]. At diets containing 0.08-0.1 ppm clinical signs of deficiency may not occur, but depletion of liver vitamin B_{12} stores cannot be excluded [4].
A concentration of 0.3-0.5 ppm (including > 0.25 ppm in a soluble form) is recommended in order to optimise the digestibility of cellulose in forages [43].

5.2 Sheep

For growing lambs, dietary requirement is estimated to be 0.11 ppm Co (DM) [61] or 0.2 mg Co/day [28]. The value of 0.11 ppm Co (DM) is also recommended for all other categories of sheep (pasture/conserved roughage) [4].

5.3 Goats

Without specific data, for all categories of goats 0.1 [26; 34] or 0.11 [1] ppm Co (DM) is recommended. By analogy of cattle, 0.2 ppm Co (DM) is recommended for high-concentrate rations (no data on ration composition given) [1].

An abnormal substance increasing Co requirements is the neurotoxin occurring in the grass *Phalaris tuberosa* [61]. However, as this grass does not occur in the Netherlands [18], this phenomenon is irrelevant under Dutch circumstances. It is not known as to what extent the two *Phalaris* grasses occurring in the Netherlands (*P. arundinacea* (in Dutch: rietgras) and *P. canariensis* (in Dutch: kanariezaad)) contain this toxin either.

6 CRITERIA TO JUDGE COBALT STATUS

6.1 Soil pH, soil cobalt and dietary cobalt concentrations

Both soil and dietary Co concentrations can assist in diagnosing Co deficiency. Soil Co concentrations should be sufficient, although Co uptake by the plants is pH-dependent [8]. Soils with pH >5.5 pose a risk for Co deficiency when cattle are fed forage grown on it [31]. On sandy soils, the Co content of the pasture depends on the pH and drainage of the soil, as shown in Table 3 [35].

Table 3. Influence of soil pH and drainage on crop cobalt content (ppm (DM)) [35]

	Soil pH		Drainage	
	5.4	6.4	good (pH 6.4)	poor (pH 6.1)
	Co concentration (ppm (DM))			
Ryegrass	0.35	0.12	0.11	0.64
Red clover	0.22	0.12	0.17	1.20

Besides this, high soil Fe or Mn concentrations can decrease crop Co contents [35].

However, the soil Co concentrations can be used to judge the Co supply to the animals. Criteria are given in Table 4 [7].

Table 4 Criteria to judge the Co content of the soil in relation to Co supply of the animals [7].

Co-content of the soil (mg/kg; 0-10 cm depth)	Conclusion	Recommended fertilization (kg Co/ha)
< 0,11	poor	0.5
0,11-0,29	marginal	0.3
> 0,29	sufficient	0

Dietary Co levels as to judge Co supply to ruminants should be interpreted carefully. Contamination of feed with soil particles increases the Co content of the feed, but absorption of Co from soil particles is poor [8; 61]. In general, 0.07-0.08 mg Co/kg DM is considered to indicate for a deficient dietary Co supply [8; 61], whereas 0.10-0.25 ppm Co is considered sufficient [45]. In grazing sheep, Co contents of the grass ranging from 0.05-0.17 ppm (DM) may elicit clinical deficiency symptoms and plasma vitamin B_{12} concentrations of 200 pmol/L. Cobalt in grass seems to be less available for vitamin B_{12} production than is Co in purified rations[14] [53]. In one case, 0.4 ppm Co (DM) appeared to be insufficient to prevent Co deficiency [31]. Feeding 0.5 ppm (DM) or more is considered to be unwarranted and wasteful [30].

6.2 Blood and tissue indicators of cobalt status

Reference values for selected indicators of Co status are given in Table 5.
Serum Co is considered not to be a reliable indicator of Co status [8]. As Co is only useful when it is incorporated in vitamin B_{12}, determining vitamin B_{12} concentrations is common to judge Co status. In sheep, this approach has been proven to be more or less satisfactory to discriminate between Co-sufficient and severely Co-deficient sheep. For the detection of mild Co deficiency, serum vitamin B_{12} determinations are less reliable. However, in cattle serum the presence of varying, but often high concentrations of corrinoids (cobalamin analogues with low or no biological potency) and binding factors rendering vitamin B_{12} unavailable for

[14] Consisting of soybean meal, casein, maize starch, cellulose and soybean oil.

analysis hampers the sound interpretation of vitamin B_{12} concentrations. Thus, considerable inter-laboratory variation can occur. In cattle, therefore, determining serum cobalamin concentrations to judge Co status is not recommended [36; 61].
Serum MMA concentrations can be used as early warning signals of imminent Co deficiency, as serum MMA values rise before appetite decreases. However, in milk-fed lambs and calves growth retardation can occur before serum MMA values rise. Moreover, in pregnant and nursing ewes MMA may be within the normal range while their lambs can suffer from vitamin B_{12} deficiency. Serum MMA concentrations have, therefore, hardly any diagnostic value for preruminant animals [61]. Finally, MMA levels can be elevated in sheep fed intensively on grain-based diets in the absence of Co deficiency. The use of MMA as a criterion to judge Co status is, therefore, recommended solely for grazing animals [33]. The usefulness of this analysis for stabled ruminants fed forage-based rations is not clear. Moreover, no clear experimental data are available to judge the value of MMA in determining Co status in cattle and goats; therefore, as yet the use of MMA for these animal species is not recommended.
Liver Co and vitamin B_{12} concentrations in both sheep and cattle only show a slightly positive correlation [37]. Therefore, this correlation will be of limited diagnostic value. Liver Co concentrations as determined by atomic absorption spectrophotometry are susceptible to high liver Fe concentrations[15]. However, the Fe effect can be overcome by chelation with cupferron and subsequent removal with chloroform before the Co determination is made [11]. Recently, the technique of Co determinations in liver has been improved, thereby enabling the use of this parameter in determining Co status (Counotte, personal communication. In general, a liver concentration of Co less than 0.1 mg/kg DM is considered insufficient for cattle and sheep [9; 45]. The liver vitamin B_{12} concentration is a sensitive indicator of Co status [61], although it is not clear as to what extent the measured vitamin B_{12} is “true” metabolically active vitamin B_{12} or its inert analogues. In general, a liver concentration of vitamin B_{12} less than 0.2 mg/ kg is considered insufficient [9; 45]. Both Co and vitamin B_{12} are equally distributed throughout the liver [42; Counotte, personal communication]. However, although taking liver biopsies is rather easy [58] the determination of vitamin B_{12} is relatively expensive and sound interpretation can be hampered by starvation (overestimation) and fatty infiltration (underestimation) [61].

6.3 Urinary FIGLU excretion

Urinary FIGLU concentration has been demonstrated to be an early indicator of Co deficiency in lambs and calves [61]. This indicator can increase 3-fold within three weeks of the beginning of a decrease in appetite [35] and again decrease to virtually zero within one week of Co treatment [50]. Sheep excreting >12 µg FIGLU/mL urine are considered deficient [35]. However, ewes not excreting FIGLU in their urine can nurse lambs which do so [46; 47; 61].

6.4 Milk vitamin B_{12} concentrations

Determinations of milk vitamin B_{12} concentrations are particularly responsive to dietary Co supply (Table 2), whereas the analytical problems associated with serum / plasma vitamin B_{12} analysis (presence of binding factors) do not occur. However, more experimental data are needed to judge the suitability of this parameter in practice.

[15] When determining Co concentrations in livers containing high Fe and low Co concentrations poor replication and obviously incorrect Co values were reported.

Table 5. Marginal bands as given for some indicators of Co / vitamin B_{12} status in weaned ruminants

Indicator	Category	Deficient	Marginal	Adequate	Ref.
Vitamin B_{12} in					
serum (pmol/L)	Cattle	29-147	184-257	294-662	[45]
		<55			[35]
		<40	40-80	>80	[61]
	Preruminant calf	<30	30-60	>60	
	Other ruminants	<336	336-500	>500	
	Preruminant lamb	<230	230-350	>350	
	Sheep	<150	150-300	>300	[62]
				>369	[30]
		<148 [a]	148-221 [a]		[35; 50]
liver (ppm)	Cattle	0.04-0.10	0.11-0.22	0.25-2.50	[9; 45]
		<0.1			[41]
		<0.11	0.11-0.19		[35]
	Sheep			>0.2	[30]
	All ruminants	<0.38	0.38-0.46	>0.46	[61]
milk (pmol/kg)	Cattle	<250	250-500		
serum MMA (µM)	Weaned ruminants	>10	5-10	<5	
Liver Co (ppm (DM))	Cattle	< 0.1		0.1-0,3	[9; 45]

[a] tentative criterion only

6.5 Conclusions

The method of choice for the determination of Co status in ruminants depends on the type of animal. In cattle, liver Co concentrations may be used, whereas liver vitamin B_{12} may be considered for additional information. Criteria are given in Table 5. Reference values for serum vitamin B_{12} concentrations in cattle are too variable to be useful in practice. In sheep, serum / plasma vitamin B_{12} determination is more reliable than in cattle. However, as plasma vitamin B_{12} determination is reported not to discriminate between subclinical and clinical Co deficiency, a combination of plasma vitamin B_{12} and plasma MMA concentrations can be used [33]. Criteria are given in Table 6. In lactating ewes, these analyses do not supply reliable information as to a sufficient vitamin B_{12} supply to the suckled lamb. For suckling animals themselves, no sufficiently reliable test for the Co status is available, although serum MMA and urinary FIGLU concentrations may provide some information. Unless proven otherwise, the values for sheep can be used for goats too.

Table 6. Criteria to distinguish between clinical and sub clinical Co deficiency in sheep (adapted from reference [33])

Plasma Vitamin B_{12}(pmol/L)	Plasma MMA (µM)	Status
	<4.6	Normal
<220	4.6 - 15.0	Subclinical deficiency
<220	>15.0	Clinical deficiency

7 DEFICIENCY

The glucose supply of ruminants largely depends on gluconeogenesis. As an adequate supply of vitamin B_{12} is vital for gluconeogenesis, this could explain that ruminants are more sensitive to Co- / vitamin B_{12} deficiency than are non-ruminants [41]. However, as in Co deficiency ruminal micro-organisms cannot convert succinate into propionate[16], both ruminal and plasma succinate concentrations increase. As succinate is the product of the reaction catalysed by methyl malonyl CoA mutase and can be used directly for glucose production, the effect of Co deficiency might be unimportant in terms of gluconeogenesis [61].
Early signs of Co deficiency are decreased appetite and growth. The loss of appetite is believed to be related to propionate accumulation in the blood due to failure of conversion into succinate. The failure to convert propionate causes increased concentrations of the intermediary methylmalonate (MMA) in blood and urine [61]. In prolonged deficiency, unthriftiness, rapid weight loss, decreased wool growth, pale skin and mucous membranes (due to normocytic, normochromic anaemia) and photosensitivity can occur. Finally, animals can die due to Co deficiency. Ewes fed Co-deficient rations in early pregnancy may give birth to few lambs. Lambs may be stillborn or less viable. Reduced milk yield of the ewe may contribute to poor lamb growth. Newborn calves seem to be less sensitive to Co deficiency during pregnancy. Impairment of fertility (decreased oestrous activity, failure to conceive, low number of offspring weaned) and increased susceptibility to disease (e.g. to *Ostertagia* and *Mycobacterium* infections) are often observed in Co-deficient animals. At pathological examination, fatty degeneration of the liver has been reported in lambs and Angora goats (ovine white liver disease, OWLD) [35; 39-41; 61; 63].
Although the Co requirement of lambs is supposed to be 0.11 ppm (DM), in grazing lambs signs of Co deficiency (OWLD, photosensitivity, low liver vitamin B_{12} concentrations) can occur when pastures contain less than 0.21 ppm Co (DM) [6; 60; 62]. However, in these cases other factors contributing to the clinical picture (e.g. fungal toxins in the grass) have been suspected [6].
In lactating dairy cows, the administration of extra Co (5.5 mg/cow/day) to a ration containing >0.2 ppm Co (DM) increased DMI, milk yield and body condition. Initial milk lactose concentrations were <4.5% and increased to >4.5% after addition of Co to the ration [31]. Unfortunately, no control group not receiving extra Co was used, so that other factors influencing milk lactose concentrations could not be excluded.

7.1 Direct measures in deficiency cases

7.1.1 Direct continuous supplementation

Cobalt can be supplemented via mineral supplements, drinking water or salt licks. The first procedure is the most convenient and economical one. Supplementation of at least 0.1 ppm Co in the concentrate should be sufficient [61]. However, addition of 0.75 ppm Co (DM)[17] to a total mixed ration containing already 0.4 ppm additional Co (DM) (from mineral supplements) resulted in increased milk yield, activity and DMI of dairy cows [31]. The use of salt licks as to supply extra Co is insufficiently reliable due to the variability in salt (and Co) consumption.

7.1.2 Direct discontinuous supplementation

Both oral supply of Co and/or parenteral supply of vitamin B_{12} can help in overcoming Co-/vitamin B_{12} deficiency in ruminants. In ruminants, only anaerobic bacteria in the rumen can use Co to synthesize vitamin B_{12}. Therefore, parenteral Co administration is senseless as Co

[16] Both the host (conversion of propionate into succinate; gluconeogenesis) and its propionic acid bacteria in the rumen (conversion of succinate into propionate; production of propionate) depend on the same Co-containing enzyme for catalysis of this reaction [61].
[17] 12 mg/cow/day at a DMI of 16 kg/day.

administered in this way does not enter the rumen in sufficient quantities [28; 61]. On the other hand, oral administration of vitamin B_{12} is much less effective than is injection because of the poor absorption of the vitamin from the gastrointestinal tract [61].
Injections of 1-2 mg vitamin B_{12} per month are recommended for deficient lambs, whereas doses of 6 mg/50 kg BW every 6 weeks improved growth of deficient beef calves. Oral Co dosing should be frequent to be effective. Oral dosing of lambs with 2 x 2 mg or 7 mg of Co (from dilute Co solutions) each week is recommended. For mature cattle, the corresponding dose is 20-70 mg of Co / week. Cobalt doses included in anthelmintic drenches may be insufficient to sustain adequate vitamin B_{12} levels in deficient areas. When Co status of ewes is marginal (or when the Co content of the grass is inadequate), Co should be supplemented throughout late pregnancy to avoid anorexia and pregnancy toxaemia. Treating lambs with oral Co supplements from 6-8 weeks of age is recommended to stimulate vitamin B_{12} formation by the lambs [61].

7.1.3 Slow release oral supplementation

Slow release boluses have been shown to supply adequate amounts of Co to both sheep and beef cows for months to years. Former types of these boluses were affected by the formation of a calcium phosphate coat, which hampered the Co release from the bolus. Newer types, such as soluble glass boluses are not susceptible to coating [61].

8 TOXICITY

8.1 General

Cobalt has a relatively low toxicity. Available evidence on Co toxicity is, therefore, scarce. Clinical signs of Co toxicity, such as decreased appetite and growth, anaemia, emaciation and debility, resemble those of Co deficiency. In toxicity cases, liver Co levels are high (20-69 ppm (DM)) [61], but tissue Co concentrations are not suitable to judge Co status [45]. No obvious pathological signs can be observed. Based on the observations that 4-10 mg Co/kg BW resulted in decreased appetite, anaemia and death in sheep and 0.9 mg Co/kg BW reduced appetite in ruminating dairy calves (17-28 weeks of age), a maximum tolerable level of approximately 30 ppm Co (DM) has been proposed [4; 10; 45]. However, in other experiments ruminating dairy calves[18] [24] and wethers[19] [17] have been demonstrated to tolerate higher dietary Co concentrations without detrimental effects on performance. No specific data on Co toxicity in goats are available[1]. For all ruminants, the NRC recommends a maximum tolerable level of 10 ppm (DM) [39; 41]. However, in the light of the above remarks this limit seems to be too low. The limit of 30 ppm (DM) seems to be sufficiently safe to protect ruminants from Co toxicity.

8.2 Direct measures in toxicity cases

In cases of suspected Co toxicity, replacing the suspected feed by a low Co feed is the only useful measure. No specific measure antagonizing Co toxicity is known.

[18] 1-40 weeks of age; 1.1 mg/kg BW.
[19] 60 kg BW; 40 ppm Co during 60 days.

9 PREVENTION

9.1 Short-term prevention strategies

Short-term prevention of Co deficiency is best performed using the oral route for Co supply, as this is much cheaper than vitamin B_{12} injections. Soluble glass boluses, which are retained in the reticulo-rumen, slowly release Co and are a convenient and safe way of supplying Co to ruminants.

9.2 Long-term prevention strategies

In deficient areas, Co (as Co salts or oxide ores) may be applied as a pasture fertilizer. The effect of a top-dressing with $CoSO_4$ on liver vitamin B_{12} levels can last up to 3-4 years [48; 59]. Depending on the soil Co status, 0.1 [8], 0.3-1.5 kg of $CoSO_4$ / ha or up to 0.5 kg Co/ha may be needed (Table 4). In the second year after application of $CoSO_4$, however, Co levels in the pasture may be approximately half of the concentrations in the first year [7; 48; 61]. Using Co fertilizers is mainly useful on sandy soils. In many cases, application of Co as a fertilizer is too expensive to be economic. To spare costs, treating only strips of pasture is recommended [61].

Table 7 Inventory of Co allowances for cattle, sheep and goats as used in some foreign countries (ppm (DM))

Country	Ref.	Allowance				
		Cattle	Ref.	Sheep	Ref.	Goat
Great Britain	[4; 61]	0.11 (maintenance of liver Vitamin B_{12} stores); 0.08-0.1 might be sufficient; 0.2 for high concentrate rations			[1]	0.11 (0.2 for high concentrate rations)
USA[a,b]	[40; 41]	0.11 (dairy cattle) 0.1 (DM; beef cattle)	[39]	0.1 slightly higher requirements for rapidly growing lambs	[38]	
Germany	[12]	0.20		?	[5]	0.15-0.20
France	[13]	0.1 (0.07 is deficiency limit)				

[a] Allowances for cattle are expressed in mg/kg feed as fed; as DM contents of the feeds are not given, allowances cannot be calculated in ppm (DM).
[b] minimum requirements.

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