Reviews on the mineral provision in ruminants (VI): CHLORINE 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.

Utrecht/Lelystad, September 2005.

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

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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
ARC		Agricultural Research Council (UK)
BW	kg	Body weight
CVB		Centraal Veevoederbureau (NL) (Central Bureau Livestock Feeding)
DLG		Deutsche Landwirtschaft Gesellschaft (G)
DM	kg	Dry matter
DMI	kg	Dry matter intake
ECF		Extracellular fluid
ha		Hectare
INRA		Institute National de la Recherche Agronomique (F)
kg		Kilogram
L		Litre
mg		Milligram
MJ		Megajoules (= 10^6 Joules)
mmol		Millimoles
mM		Millimolair
NEl	MJ	Net Energy lactation (G)
NRC		National Research Council (USA)
SD		Standard deviation
vol	L	Volume
wt	Kg	Weight

1 PHYSIOLOGICAL FUNCTIONS OF CHLORINE

Chlorine (Cl) is the principal anion in extracellular fluid (ECF) and together with bicarbonate (HCO_3^-), it almost completely balances electrically with the present sodium (Na). Therefore, Cl also is a major determinant of the osmotic pressure of ECF and is of great importance in maintaining water balance in animals. Variations in osmolarity of the ECF are usually the result of changes in cation rather then anion concentrations (23). The Cl concentration of ECF is subject to more variation than that of Na, because HCO_3^- can exchange for Cl, thereby affecting systemic acid-base balance. Chlorine is also involved in respiration. Diffusion of carbon dioxide into erythrocytes is followed by dissociation of carbonic acid due to the presence of carbonic anhydrase. The bicarbonate ions diffuse readily from the erythrocytes into the plasma and electrical neutrality is maintained by the accompanying diffusion of Cl from plasma into the erythrocytes. This transfer of Cl ions is known as the chloride shift. These processes are reversed in the lungs (35). Furthermore, Cl is the chief anion of the gastric secretions in the abomasum (21) and together with the hydrogen ion, it acidifies the stomach contents which is important for protein digestion (6).

2 DISTRIBUTION OF CHORINE BETWEEN TISSUES

Typical Cl concentrations in ECF are 104-105 mM and 108-117 mM in plasma and interstitial fluid respectively, while intracellular concentrations are rather low; i.e. 4 mM (23, 36).

3 CHLORINE METABOLISM

3.1 Absorption

3.1.1 Site and mechanism of Cl absorption

As far as we know, there are no studies available with cannulated animals, which provide quantitative information about the main site of Cl absorption. Apart from dietary origin, Cl also enters the rumen together with saliva. The Cl concentration of saliva is about 6.5 mmol/L, but it may range from 3 to 10 mmol/L (4).

It is generally accepted that most of the Cl that is absorbed in the rumen is actively transported across the rumen epithelieum into the mucosal - serosal direction either directly or indirectly coupled with Na (29). It is currently believed that the electroneutral mechanism for Na and Cl transport may be described as a dual exchange; i.e. Na/H working in parallel with Cl/HCO_3 (10, 18, 27, 41). In contrast to rumen epithelium, Cl is secreted by the epithelial cells of the omasum (13, 14, 29, 44) and it is probably associated with the absorption of bicarbonate (14). Chloride is also secreted into the gut contents by the parietal cells of the abomasum (13, 44) together with hydrogen, which is derived from H_2CO_3. The concurrent HCO_3^- produced, is exchanged with Cl^- at the basolateral membrane to maintain electroneutrality of the blood (3). It was estimated by Sklan and Hurwitz (44) that only 8.7 % of Cl intake was absorbed in the ileum, while 81.3 % of the Cl ingested was estimated to be absorbed in the large intestines of sheep. This is in contrast with other observations in sheep by Pfeffer et al. (cited by Martens (28)) that Cl mainly disappeared in the distal part of the ileum. Nevertheless, both observations (28, 44) agree in that Cl is efficiently absorbed distal to the duodenum. Chloride may be transported by several mechanisms such as Na/Cl and Na/K/2Cl co-transporters or by Cl/HCO_3 exchangers (20, 28).

3.1.2 Cl absorption in relation to Cl source

Little is known about the availability of Cl from several feedstuffs. In general, Cl is highly absorbed, the ARC (2) gives an absorption coefficient of 85% while INRA(19), NRC (34) and DLG (12) estimate values of 90, 90 and 95% respectively. With respect to dietary supplements, Henry (22) indicates that the availability of Cl from NH_4Cl and KCl may be 95% relative to that from NaCl. Further information is yet not available.

3.2 Excretion

The concentration of Cl in the ECF tends to be regulated secondarily to regulation of the Na and HCO_3 in ECF. If excess Na is excreted by the kidney, Cl usually accompanies it. If, because of an alkalotic condition, the plasma HCO_3 rises, an equivalent amount of Cl is excreted in order to maintain electroneutrality of the ECF (23). The process of Cl reabsorption in the proximal tubulus of the nephron is passive (20). Thus, low plasma Cl concentrations facilitate the rate of Cl transport from the filtered load into plasma. Indeed, when lactating cows were fed a Cl deficient ration, plasma Cl concentrations dropped and the urinary excretion of Cl reduced to almost zero (< 35 mg/day) within 1 week (15).

4 CHLORINE REQUIREMENTS

4.1 Dairy cows

4.1.1 Maintenance

Currently, there is no Dutch estimate for the maintenance requirement of Cl of dairy cows (5). The German estimate for the maintenance requirement of Cl is entirely based on the faecal excretion of Cl (Table 1). A value of 0.71 g Cl/kg faecal water was adapted by the DLG as an estimate for the inevitable faecal Cl losses (Table 1). Assuming a digestibility of the dry matter of 65% (a value corresponding to a NEl of 5.2 MJ/kg DM (=equivalent to 750 VEM) and a dry matter content of faeces of 15%, each kg of dry matter ingested yields 2 kg of faecal water. In order to maintain energy balance, a cow with a body weight of 600 kg requires 6.6 kg DM of feed with an energy content of 5.2 MJ NEl (roughage), resulting in a faecal loss of 9.4 g of Cl/day; i.e. 15.6 mg/kg BW. Dermal (including sweat) and urinary Cl losses are not estimated by the DLG (12). The German estimate for obligatory faecal Cl losses is about 2 times higher than that estimated by the ARC (Table 1); i.e. 7.9 mg/kg BW (Table 1).

The value adapted by the ARC, is derived from the pooled regression (4 studies) of faecal output of Cl on Cl intake; i.e. faecal Cl (g/day) = 0.154 x Cl intake (g/day) + 3.96 (r = 0.67, p <0.001). Thus, based of this regression, faecal Cl loss at zero Cl intake appears to be 3.96 g/day. Then, the intercept was divided by the assumed BW of the animals (500 kg) resulting in a value equal to 7.9 mg/kg BW (2). The French (19) estimate the endogenous losses of Cl by multiplying the total endogenous Na losses by a factor 1.5, thereby probably assuming that the endogenous losses of Na and Cl are excreted in 1 : 1 ratio (molar basis). The value of 22.5 mg/kg BW has been adapted by the NRC for dairy cows (Table 1). Essentially, the ARC estimate for the net Cl requirement of maintenance was also adapted by Todd (45) when mineral allowances in ruminant livestock were reviewed. Arbitrairly, we suggest to adapt a tentative value of 8.7 mg Cl/kg BW as the net maintenance requirement for dairy cattle i.e. the sum of faecal losses (7.9 mg/kg BW) and dermal/sweat losses (0.8 mg/kg BW, (2)).

4.1.2 Pregnancy

The net requirement for pregnancy set by the ARC (Table 2) was calculated on the basis of the following formula:

$$\text{Cl content of foetus and adnexa (g)} = 0.025 \times \text{Birth weight} \times 10^{4.15 - 5.54*(EXP(-0.00353*D))}$$

D = days from conception in the range of 141 to 281 (=parturition)

Assuming a birth weight of 44 kg, Cl retention in foetus and adnexa was calculated to be 1.2 and 1.9 g/day during the second an first month pre partum, respectively. The net requirement for pregnancy set by the NRC (Table 2) is based on the Na accretion rate of products of conception, which were derived from slaughter experiments of House and Bell (24). When assuming that relative proportions of Cl and Na in the foetus and in the newborn calf are similar to that observed by the ARC (2), the net Cl requirement for pregnancy was set at 1.0 g/day from 190 days of gestation by the NRC (34). Inserting the same time interval for pregnancy in the formula provided by the ARC (2) yields a similar value, i.e. 1.1 g Cl/day. The net requirements for pregnancy set by the DLG (12) are derived from the Cl content of newborn calves and are lower than those estimated by the ARC (2). The net Cl requirement for pregnancy is not estimated by INRA(19). Currently, we arbitrarily suggest to adapt the formula provided by the ARC (2) to calculate the Cl requirement of the gravid uterus during gestation.

4.1.3 Growth

The value adapted as the Cl requirement for growth used by the NRC (34) was taken from INRA (19) and none of the two councils provides any further information. The Cl requirement for growth has been set at 1.0 g/kg growth by the ARC (2). This value is based on the body content of Cl of castrated beef Shorthorn and Hereford males. The estimated Cl requirement for growth provided by the DLG (12), is also based on whole body Cl contents of young steers (no further information). Because there is no discrepancy between the different councils with respect to their estimate of the Cl requirement for growth, we suggest to adapt the value of 1.0 g/kg growth as the net Cl requirement for growth.

4.1.4 Milk production

The estimated Cl concentration in milk varies from 1.0 to 1.3 g/L (Table 2). The lower estimate agrees with that provided by Adrian (1), i.e. 0.95 g/L. The origin of the French (19) and German (12) estimates for the Cl content of milk is not further specified. The ARC (2) value for the Cl content of milk is derived from one study. However, more recent results from studies by Delaquis and Block (8, 9), Shalit et al. (42), Silanikove et al. (43), Sanchez and Beede (37), Sanchez et al. (38), Coppock et al. (7) and Fettman et al. (16) show group mean Cl concentrations in milk ranging from 0.62 to 1.3 g/L, with an overall mean content of 0.9 g Cl/L milk, which is associated with a combined within- and between animal variation of 26% (coefficient of variation). When all values referring to the Cl content of milk are combined, the Cl content of milk is 1.0 g/L. Therefore it is suggested to adapt this value for calculating the Cl requirement due to milk production.

4.2 Beef cattle

4.2.1 Maintenance

The net maintenance requirements set by the ARC (2) for beef cattle are similar to those of dairy cattle with the exception that a somewhat higher value (reason unknown) was used to estimate the dermal Cl losses (Table 1). The French estimates (19) for the net maintenance requirements of Cl for beef cattle are equal to those for dairy cattle and is based on Na requirement (see section on dairy cows). Because specific further information with respect to the maintenance requirement of Cl is not available, we consider it opportune to adapt the approach of the ARC (2). Thus, the suggested net Cl requirement for maintenance in beef cattle is estimated to be 8.9 mg/kg BW; i.e. the sum of faecal losses (7.9 mg/kg BW) and dermal/sweat losses (1.0 mg/kg BW (2)).

4.2.2 Growth

Specific factorial estimates concerning net Cl requirement for growth in steers are not provided by any of the listed councils (Table 2). However, the Cl requirements for growth of dairy cattle provided by the ARC are also based on observations in beef cattle (see section about dairy cows). Therefore, we suggest to adapt the same value for growth in beef cattle; i.e. 1.0 g Cl/kg growth. Factorial estimates with respect to pregnancy and milk (suckling cows) are not given by any of the listed councils.

4.3 Sheep

4.3.1 Maintenance

The estimate for the faecal endogenous Cl losses of sheep provided by the ARC (2) is not based on sheep data, but is derived from the previous mentioned regression calculated for cows (section 4.1.1.). The French estimate of the Cl requirement in sheep is based on Na (see previous sections). As far as we know no further data on Cl balances in sheep (46) have been published. Arbitrarily, we suggest to adapt the value of the ARC (2) to estimate the net Cl requirement in sheep; i.e. 8.7 mg/kg BW; i.e. the sum of faecal losses (7.9 mg/kg BW) and dermal losses (0.8 mg/kg BW).

4.3.2 Pregnancy, growth and milk production

The net requirement for pregnancy is not provided by any of the listed councils (Table 2). Since there appear to be no experimental data on Cl retention during gestation, a practical approach might be to use the estimates of Cl retention in pregnant cows after adjusting birth weights. This approach erroneously implies, amongst others, that the foetus to adnexa ratio is similar in cattle and sheep (2), but the practical consequence is not known. Thus, under the assumption of a total birth weight of 8 kg, it was decided to use a value 0.3 g Cl/day during the last 8 weeks of pregnancy. Growth and milk production

With respect to the net requirement of Cl for growth and milk production, there are no considerable differences between the ARC and INRA and (Table 2). Therefore, we suggest to adapt a value of 0.8 g/kg growth for sheep with BW ranging from 4 to 45 kg and a value of 0.7 g/kg growth for growing sheep with BW > 45 kg. With respect to the Cl content of milk, we suggest to adapt the mean of the two listed values in Table 2; i.e. 0.9 g Cl/L.

4.4 Goats

Estimates about the inevitable Cl losses (Table 1), net Cl requirements for growth, pregnancy and milk production (Table 2) in goats are not provided by any of the listed councils. However, Jeness (25) indicated that the mean Cl content of goat milk was 1.6 g/L (range : 1.2 to 2.0 g/L). Thus, we suggest to adapt a value of 1.6 g Cl/L to calculate the Cl requirement for milk production in dairy goats. With respect to the estimate of the net Cl requirements for maintenance and growth, we arbitrarily suggest to adapt the value used for cattle because of the incorporation of dermal losses (sweat).

4.5 Coefficient of absorption

As far as we know, data based on radioisotope studies with Cl to assess the efficiency of true absorption of Cl are not available. The ARC (2) arbitrarily adapted a value of 85 % for the true absorption of Cl, which is somewhat lower than the values taken by NRC (34), INRA (19) and the DLG publication (12) concerning dairy cows (Table 3); i.e. 90, 90 and 95%, respectively. Finally, differences between species (cattle vs. sheep) in the ability to absorb Cl are not reported by the CVB, NRC and INRA. Only the ARC mentions specifically that they adapted the same coefficient of Cl absorption for both cattle and sheep. We arbitrarily suggest to adapt a value of 90% as the true absorption coefficient of Cl for cattle, sheep and goats.

4.6 Conclusion

The following equation can be used to calculate the gross Cl requirement in the various ruminant species:

$$\mathbf{C} = \frac{\mathbf{100 \times (a + (kg\ milk \times b) + c + d)}}{\mathbf{A_{Cl}}}$$

in which C = required dietary Cl concentration (g/ day)
A_{Cl} = true Cl absorption (%) (assumed to be 90%)

a = maintenance requirement (g/day; 8.7 mg/kg BW for dairy cattle, sheep and goats and 8.9 mg/kg BW for beef cattle)
b = Cl content of milk (1.0 g/L for dairy cattle, 0.9 g/L for sheep and 1.6 g/L for goats)
c = Cl content of growing tissues (g Cl/kg growth; 1.0 g/kg for dairy and beef cattle and goats; 0.8 g/kg for sheep)
d = amount of Cl needed for pregnancy (g/days; for dairy cattle the formula given by the ARC, given in paragraph 4.1.2, is used resulting in the following values: 1.3 and 2.0 g/day 8 - 3 w and 3 – 0 w pre partum, respectively; for sheep and goats the value is 0.3 g/d for the last 8 weeks of gestation)

4.7 Allowances

In the reports on Na- and K-metabolism and requirements in ruminants of CVB (40)(39), a safety factor of 1.3 was used to calculate the Na- and K allowance for lactating animals (cows, sheep and goats). The use of a safety factor was considered opportune in the light of the reported variations in both the Na and K content of milk. Because the reported values of the Cl content of milk also show considerable variation, it was decided to adapt a safety margin of 1.3 also when calculating the Cl requirements in lactating animals.

5 CLORINE DEFICIENCY

In experimentally induced Cl deficiency in calves (30), animals became anorexic and lethargic, while mild polyuria and polydipsia were also observed. In later stages of deficiency, severe eye defects occurred and all animals died after 24-46 days of depletion. Furthermore, plasma Cl could not be maintained and values dropped from 96 to 31-35 mmol/L, just before the animals died. The drop in plasma Cl concentrations was accompanied by a secondary alkalosis and hypokalemia. The secondary drop in plasma K concentrations can be explained by diffusion of potassium ions into body cells thereby electrically balancing the release of hydrogen ions from body cells (36). Indeed, Fetmann et al. (16) observed a primary hypochloremia combined with a secondary hypokalemic metabolic alkalosis in early lacating cows fed a Cl-deficient ration. Furthermore, in lactating cows, feed intake and milk production may also be negatively affected by a deficit of dietary Cl (1 g Cl/kg of DM) (16, 17).

6 CHLORINE INTOXICATION

From a practical point of view, the toxicity of Cl is synonymous with salt (Na) toxicity (See Na metabolism in ruminants) (46)

7 CHLORINE STATUS

Based of the present information, Cl status may simply be assessed by plasma Cl and K concentrations. According to Underwood and Suttle (46) plasma Cl concentrations < 85 mmol/L are indicative for Cl deficiency. Furthermore, Fettman et al (17) observed serum K concentrations < ± 3.6 mmol/L in Cl deficient animals. Thus, it is suggested that the combination of low plasma Cl and K concentration is highly indicative for Cl deficiency. Finally, urinary Cl concentrations are extremely low (< 2-5 mmol/L) after feeding of a Cl deficient ration (46).

Table 1. Summary of estimates of endogenous Cl losses (expressed in mg/kg BW, unless otherwise noted).

	Endogenous/inevitable losses			
	Dermal/Sweat	**Fecal**	**Urine**	**Total**
Dairy cows				
CVB (5)	not given	not given	not given	not given
ARC (2)	0.8[1]	7.9	negligible	8.7
DLG (12)	not given	not given	not given	0.71 g/kg faecal water[2]
NRC (34)	not given	not given	not given	22.5
INRA (19)	not given	not given	not given	22.5
Beef cattle				
CVB (5)	not given	not given	not given	not given
ARC (2)	1.0[3]	7.9	negligible	8.9
DLG (11)	not given	not given	not given	not given
NRC (33)	not given	not given	not given	not given
INRA (19)	not given	not given	not given	22.5
Sheep				
CVB (5)	not given	not given	not given	not given
ARC (2)	negligible	7.9	negligible	7.9
NRC (32)	not given	not given	not given	not given
INRA (19)	not given	not given	not given	22.5
Goats				
CVB (5)	not given	not given	not given	not given
ARC (2)	not given	not given	not given	not given
NRC (31)	not given	not given	not given	not given
INRA (19)	not given	not given	not given	not given
Kessler(26)	not given	not given	not given	not given

[1] Temperate conditions (no further specifications).
[2] Calculated on the assumption that faecal water contains 20 mmol Cl/L.
[3] No specifications provided.

Table 2: Summary of estimates of net Cl requirements for foetal retention, growth and milk.

	Gravid uterus (g/d)	Growth (g/kg gain)	Milk (g/L)
Dairy cows			
CVB (5)	not given	not given	1.2
ARC (2)	1.2[1] (8-4 weeks ante partum)	1.0 (75-500 kg BW)	1.13[2]
	1.9[1] (4-0 weeks ante partum)		
DLG (12)	0.8 (6-4 weeks ante partum)	1.0	1.3
	1.0 (3-0 weeks ante partum)		
NRC (34)	1.0[3] (190-270 days of gestation)	1.0 (150-600 kg BW)	1.15
INRA (19)	not given	1.0 (150-600 kg BW)	1.0
Beef Cattle			
CVB (5)	not given	not given	not given
ARC (2)	not given	not given	not given
DLG (11)	not given	not given	not given
NRC (33)	not given	not given	not given
INRA (19)	not given	not given	not given
Sheep[4]			
CVB (5)	not given	not given	not given
ARC (2)	not given	0.8[5](4-45 kg BW)	1.1
NRC (32)	not given	not given	0.75
INRA (19)	not given	0.7 (10-50 kg)	not given
Goats			
CVB (5)	not given	not given	not given
ARC (2)	not given	not given	not given
NRC (31)	not given	not given	not given
INRA (19)	not given	not given	not given
Kessler (26)	not given	not given	not given

[1] Calf with birth weight of 44 kg.
[2] British Friesian cows
[3] Not measured but estimated on the basis of Na accretion as determined by House and Bell (24).
[4] Chlorine accretion due to wool production is not provided by ARC (2).
[5] Estimated on the basis of data from cattle.

Table 3: Summary of estimates for the coefficient of Cl absorption (% of intake)

Reference	True absorption
CVB (5)	not given
ARC (2)	85
DLG (12)	95
DLG (11)	not given
NRC (34)	90
INRA (19)	90
Kessler (26)	not given

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