Reviews on the mineral provision in ruminants (IX): COPPER METABOLISM AND REQUIREMENTS IN RUMINANTS

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COPPER 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
Chair of the COMV

Dr. M.C. Blok
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, Dr. ir. A.W. Jongbloed 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
A_{Cu}	%	Absorption coefficient for Cu
ARC		Agricultural Research Council (UK)
BW	kg	Body weight
CuLys		Cu lysine
CuMet		Cu methionine
CVB		Centraal Veevoederbureau (NL) (Central Bureau Livestock Feeding)
DLG		Deutsche Landwirtschaft Gesellschaft (G)
DM	kg	Dry matter
DMI	kg/day	Dry matter intake
h		Hour
ha		Hectare
INRA		Institut National de la Recherche Agronomique (F)
IU		International Units
L		Litre
MJ		Megajoules (= 10^6 Joules)
mmol		Millimoles
mM		Millimolair
µg		Microgram
NRC		National Research Council (USA)
ppm	mg/kg	Parts per million

1 FUNCTIONS OF COPPER IN THE BODY

Copper is an essential component of many enzymes. As such it is involved in the synthesis of connective tissues (lysyl oxidase, formation of cross links in collagen and elastin) and iron transport and haemoglobin formation (caeruloplasmin). Moreover, it is involved in the electron transport during aerobic respiration (cytochrome oxidase) and the protection of the body against oxygen radicals (superoxide dismutase). Finally, Cu is needed for the production of melatonin (tyrosinase) [98].

2 COPPER DISTRIBUTION

In the body, the liver is the main site of Cu storage. Normal concentrations roughly range from 100-400 ppm (DM) [106]. In the newborn lamb, approximately 50% of the total body Cu is located in the liver [11]. Outside the liver, the highest concentrations are found in the heart and kidney (11.1 and 12.9 ppm (DM), respectively). As the main contributors to carcass weight (muscle, fat and bone) are relatively poor in Cu, Cu concentrations in total extrahepatic tissues are low (2 ppm (DM; sheep) 1.3 ppm (DM; cattle, assuming the DM content of the extrahepatic tissues to be 60% [77]). Besides this, a certain amount of Cu is present in the fleece [140].

3 COPPER ABSORPTION

3.1 Introduction

Only part of the dietary Cu is absorbed from the intestine. The gross amount of dietary Cu taken up from the intestine is referred to as “true absorption”. “Apparent absorption” is defined as the difference between the amount of Cu in the ration and the amount present in the faeces. However, apparent absorption is often not sufficiently informative as it does not account for the amount of the endogenous excretion of Cu. Part of the Cu that has been (truly) absorbed from the intestine, and / or mobilized from body stores, is secreted into the intestine. Part of this endogenous secreted Cu will be reabsorbed. Another fraction, however, is excreted, and is called the endogenous faecal Cu loss. True absorption can be calculated from the apparent absorption when the faecal endogenous loss is known [140].

The faecal endogenous loss can be calculated using a radioisotopic dilution technique. After parenteral injection of a Cu radioisotope (e.g. ^{64}Cu) into an animal, total collection of faeces and total excretion of both radioisotope and stable (non-radioactive) Cu isotope, the endogenous faecal Cu loss can be calculated using the equation:

$$\text{endogenous faecal Ca (mg)} = \frac{\text{specific activity of faeces (counts/g)} \times \text{total faecal Cu (mg)}}{\text{specific activity of endogenous Cu (counts/g)}}$$

in which specific activity is the ratio of radioisotopic to stable Cu.

The specific activity of endogenous Cu is assumed to be predictable from samples taken from an accessible pool with which the endogenous Cu is in equilibrium (e.g. plasma or urine). The true Cu absorption coefficient can be calculated using the equation [140]:

$$\text{true Cu absorption coefficient} = \frac{\text{Cu intake(mg)} - (\text{faecal Cu (mg)} - \text{endogenous faecal Cu (mg)})}{\text{Cu intake (mg)}}$$

in which the true Cu absorption coefficient has no unit. When Cu absorption has to be expressed in % units, the result from the equation has to be multiplied by 100. For a more detailed description including (dis)advantages of this method, see reference [140]. However, outcomes of this method to calculate faecal endogenous Cu losses can differ by a factor of three [127].

In man and rats, intestinal Cu absorption starts in the stomach [29]. In calves, however, net Cu secretion occurs in the abomasum, whereas net Cu absorption occurs along the rest of the intestinal tract [62]. Copper uptake by hamster and rat intestinal cells is saturable [22]. In man, a putative Cu transporting protein (hCTR1) may transport Cu into the intestinal cells, whereas MNK (an ATPase, defective in patients with Menkes disease which accumulate Cu in intestinal cells) is thought to transport Cu from the intestinal cells into the portal circulation [103]. Within intestinal cells, Cu can be bound to thionein to form metallothionein. In rats, Cu induces less metallothionein formation than Cd or Zn do [22]. In Suffolk-Western Whiteface x Dorset sheep fed diets containing either 2.2, 11.3 or 47.0 ppm Cu, no induction of metallothionein in intestinal cells could be demonstrated. No Cu was associated with the metallothionein fraction of the cytosol of intestinal cells at either dietary Cu level [110]. Possibly this indicates for poor regulation of Cu intake and the relatively high Cu accumulation in tissues of certain sheep breeds. Moreover, uptake of dietary Cu depends more on the concentrations of certain ration components interacting with Cu absorption (see 3.2) than on the dietary Cu concentration [140].

In the portal blood Cu is mainly bound to albumin and / or amino acids (e.g. histidine). This binding is not very tight, thereby enabling Cu to be easily released from this compartment into the liver but also into extrahepatic tissues. A large part of the portal Cu is taken up by the liver during the first passage (man ± 80%, rat ± 50%). This Cu is subsequently released from the liver as caeruloplasmin [29]. In man, this protein has a half-life of 4-7 days [109]. In cattle, approximately 80% of systemic plasma Cu is bound to caeruloplasmin [137]. The remainder is mainly bound to albumin and amino acids (histidine) [109]. In other animal species this value can even increase to 90% [29]. The bond of Cu to caeruloplasmin is relatively tight. Copper bound to caeruloplasmin probably acts as a Cu source for peripheral tissues, although Cu bound to albumin and amino acids also may be important [109]. There seems to be no exchange of Cu between albumin, amino acids and caeruloplasmin [29]. Moreover, albumin seems to have different binding sites for Cu and Zn. Thus, competition of Cu and Zn for binding sites of albumin during release from the intestine seems to be of no practical importance [29].

When dietary Cu supply exceeds requirements, Cu is mainly stored in the liver and subsequently released when plasma Cu declines [140]. *In vitro* Cu uptake by the liver is saturable and temperature dependent. The ratio between albumin- and amino acid-bound Cu could affect the amount taken up by the liver. Copper uptake by the liver seems to be a passive transport using a carrier [29].

In cattle, Cu efficiently crosses the placenta [128]. The Cu status of newborn calves depends, therefore, on the Cu supply to the dam [27]. In neonatal calves hepatic Cu concentration is usually higher than in adult animals [44; 64]. During pregnancy, increasing foetal liver Cu concentrations can be associated with decreasing dam liver concentrations, even when dam liver Cu concentrations are suggestive of deficiency (<25 ppm Cu (DM)) [44]. However, in other reports foetal and dam liver Cu concentrations were positively correlated [46], or foetal liver Cu did not increase during pregnancy [2].

3.2 Copper absorption from different sources

In practice, the amount of Cu present in feed ingredients of plant and animal origin is generally not taken into account in feed formulations, due to enhanced costs to analyse them. Moreover, no data are available with regard to the absorption of Cu from these feeds in ruminants. Therefore, only Cu absorption from different mineral sources can be compared here.

Besides its function as a vital trace element for animals, Cu poses a potential environmental hazard. Therefore, in order to minimize Cu losses to the environment, only the best available sources should be used as supplements for livestock rations. To evaluate differences in bioavailability of Cu from different mineral sources, scientific literature was reviewed. *In vivo* nutritional experiments with cattle, sheep and goats were included in this search. Furthermore, only those experiments were used that compared at least two different sources. For Cu, sources were grouped as $CuSO_4$ and other inorganic sources, and compared with organic Cu complexes. As CuO has been proposed to be abandoned by EU legislation, no attention has been paid to this source except for use as CuO needles (see 8). CuLys and CuMet are cupric (Cu^+) chelates of lysine and methionine (C. Rapp, Zinpro Corp.).

3.2.1 <u>Cattle</u>

Ward et al. [144] observed higher gain (1.5 vs. 1.2 kg/day) and better feed:gain ratio (2.4 vs. 2.9 kg/kg) during the first 21 days of a 98-day experiment in steers (Angus and Angus x Hereford, 218 kg BW) fed 5 ppm additional Cu from either $CuSO_4$ compared with CuLys. This difference was still significant when 5 ppm Mo and 2 g S/kg were added to the diet (1.7 vs. 1.4 kg/day and 2.2 vs. 2.6 kg/kg for $CuSO_4$ and CuLys, respectively; Mo was added as $Na_2MoO_4.2H_2O$ and S was added as feed-grade $CaSO_4$). However, during the entire 98-day trial, the differences were not significant. The basal diet (maize silage-soybean meal-based) contained 6.2 ppm Cu, 0.8 ppm Mo, and 1.7 g S/kg.

Comparing CuLys and $CuSO_4$ as Cu sources for Brahman x Hereford heifers (13.5 months of age; 301 kg BW) consuming a maize-soybean meal-cottonseed hulls-based diet containing (DM) 5 ppm Cu, 1000 ppm Fe, 5 g S/kg, and 5 ppm Mo for 71 days, Rabiansky et al. [107] found no differences in hepatic Cu concentrations between the two sources. After feeding lower Fe and S concentrations (50 ppm Fe, 1.6 g S/kg (DM), Mo not given) for another 97 days, only CuLys caused higher liver Cu concentrations than $CuSO_4$ at a level of 16 ppm Cu (approximately[5] 110 vs. 75 ppm Cu in liver DM, respectively). In contrast, adding 5 ppm Cu (DM) of either $CuSO_4$, $CuCO_3$, or Cu proteinate (Cu valence unknown) to a maize silage-soybean meal-based diet (6.3 ppm Cu, 3.8 g S/kg, 6.9 ppm Mo (DM)) of Cu-depleted Angus heifers (292 kg BW) revealed declining plasma Cu concentrations with time in animals fed $CuSO_4$ (-4.7 µM). The animals fed additional $CuCO_3$ or Cu proteinate maintained plasma concentrations, whereas the heifers fed additional Cu proteinate had smaller decreases in liver Cu (-8.2 ppm (DM) vs. -23.9 to -28.7 ppm (DM); initial liver Cu concentration was 29.7-40.5 ppm (DM)) [145]. No differences were observed in performance. When Mo content of the diet was reduced to 1.9 ppm, Cu proteinate and $CuSO_4$ had a similar influence on Cu metabolism. In a similar experiment (basal diet contained 7.3 ppm Cu, 2.8 g S/kg and 3.0 ppm Mo (DM) [145]) heifers receiving 50 mg/head/day $CuCO_3$ had lower liver Cu concentrations (27.7 ppm (DM)) than those receiving either $CuSO_4$ (47.5 ppm (DM)) or Cu proteinate (44.6-49.1 ppm (DM)).
In Cu-depleted beef calves (Simmental, Charolais, Angus; 207 kg BW), no difference in parameters of Cu metabolism (plasma Cu, caeruloplasmin activity, ruminal soluble Cu) could be observed when either $CuSO_4$ or CuLys was fed at a level of 13.6 ppm compared with control animals consuming a maize silage-soybean meal-based diet containing 5.8 ppm Cu [71]. Similarly, when feeding 26 mg Cu from either Cu proteinate or $CuSO_4$ as a grass hay/concentrate diet (grass hay containing 1.0 ppm Cu and 5 ppm Mo) for Holstein calves (>12 weeks of age), Kincaid et al. [74] found higher liver Cu concentrations (325 vs. 220 ppm (DM)) and plasma Cu concentrations (13.7 vs. 11.8 µM) in calves receiving Cu proteinate than in those receiving $CuSO_4$. However, as mentioned by Underwood and Suttle [140], neither Kincaid et al. [74] nor Ward et al. [145] properly accounted for the large differences in initial Cu status (covariance analysis). Therefore, the relatively favourable effects of Cu-proteinate as demonstrated by these authors might be doubtful.

3.2.2 Sheep

Ivan et al. [63] found similar final liver Cu concentrations when lambs (28-42 kg BW) were fed either 5 or 10 ppm (DM) of additional Cu from either $CuSO_4.5H_2O$ or $CuCl_2.2H_2O$ added to a ration of 40% grass hay, 15% soybean meal, 10% wheat, 33% chopped whole-plant maize, 1% dry molasses and 1% mineral-vitamin supplement (the basal diet contained 8.6 ppm Cu (DM)). After 132 days on experimental diets, final liver Cu concentrations were 486 vs. 513 (5 ppm (DM)) and 589 vs. 598 µg/g DM (10 ppm (DM)) for sulphate and chloride, respectively.
Suttle and Brebner [134], using Suffolk-cross sheep (10 months of age, 44 kg BW), could not demonstrate significant differences between $CuSO_4.5H_2O$ and a CuLys complex (CuPLEX 100, Zinpro Animal Health Ltd.). Sheep were fed 4.0 kg DM of a diet consisting of (%) whole oats (90.8), urea (1.8), Ca sulphate (1.4), Na bicarbonate (1.8) and KCl (0.5) with trace elements and vitamins. The basal diet contained 3.5 ppm Cu (DM) and was either not supplemented or supplemented with one of the Cu sources to obtain total Cu concentrations (ppm (DM)) of 9.8, 8.4, 14.6 and 13.2. At the end of the 28-day trial the final hepatic Cu concentrations appeared not to be significantly affected by Cu source.
In an experiment with wether lambs (40 kg BW), the addition of 180 ppm Cu from either CuLys or $CuSO_4.5H_2O$ to a maize/soybean meal/cottonseed hulls diet (9.8 ppm Cu (DM)) for 10 days resulted in slightly lower liver Cu concentrations in the CuLys group [84]. Initial liver

[5] Exact values were not given; values were depicted only in a graph.

Cu concentrations were 313 vs. 338 ppm (DM) and final liver Cu concentrations were 511 vs. 573 ppm (DM) for the CuLys and $CuSO_4$ groups, respectively.

3.2.3 Goats

As yet, no information on differences between Cu bioavailability from different mineral sources for goats is available.

3.2.4 Discussion and conclusions

Results of Cu bioavailability trials in cattle and sheep are summarized in Table 1.

Table 1 Summarized results of bioavailability trials with different Cu sources for cattle and sheep

Ref.	Category	N	Sources used	Response criteria	Bioavailability
[144]	fattening steers	21	$CuSO_4$, CuLys	performance	$CuSO_4$ > CuLys (first 21 days) $CuSO_4$ = CuLys (entire 98-day period)
.[107]	heifers	8	$CuSO_4$, CuLys	liver Cu	$CuSO_4$ = CuLys (high Fe, S, and Mo) $CuSO_4$ < CuLys (low Fe and S)
[145]	beef heifers	5-6	$CuSO_4$, $CuCO_3$, Cu proteinate	plasma and liver Cu	$CuSO_4$ < $CuCO_3$, Cu proteinate (high Mo) $CuCO_3$ < $CuSO_4$, Cu proteinate (low Mo)
[71]	beef calves	6-7	$CuSO_4$, CuLys	plasma Cu, CP[a], ruminal soluble Cu	$CuSO_4$ = CuLys
[74]	calves	15	$CuSO_4$, Cu proteinate	plasma and liver Cu	$CuSO_4$ < Cu proteinate
[63]	lambs	10	$CuSO_4$, $CuCl_2$	liver Cu	$CuSO_4$ = $CuCl_2$
[134]	sheep	5	$CuSO_4$, CuLys	liver Cu	$CuSO_4$ = CuLys
[84]	wether lambs	14	$CuSO_4$, CuLys	liver Cu	$CuSO_4$ = CuLys

n = number of animals/group; [a] CP = caeruloplasmin activity

Although evidence is not fully in agreement with each other, in the presence of low Mo concentrations in the ration (< ± 2 ppm (DM)) the differences in bioavailability between the different Cu sources for cattle seem to be minor. Then, the cheapest and most convenient Cu source can be fed. However, as summarized by Ward et al. [145], in the presence of higher Mo concentrations (± 5-7 ppm (DM)), Cu proteinate may be more advantageous. As yet, the reason for this difference is not clear as no data are available on the composition of Cu proteinate. As $CuSO_4$ contains S (sulphate being reduced to sulphide [99]), mainly a sufficient amount of Mo is required to evoke a Cu x Mo x S interaction, thereby rendering Cu from $CuSO_4$ less available. Besides this, $CuCO_3$ is associated with lower liver Cu concentrations and seems to be less available than $CuSO_4$ to cattle. Finally, in one experiment bioavailability of Cu chloride for cattle has been reported to be 115% when compared with $CuSO_4$ [7].
However, although Cu absorption from Cu proteinate may be somewhat higher than from $CuSO_4$, $CuSO_4$ may be the cheapest and most convenient source for Cu supply to cattle.
The scarce evidence on bioavailability of different Cu sources for sheep does not reveal any superior Cu source, compared to $CuSO_4$, for use in these animals. As no experimental evidence is available, no separate conclusions can be drawn on differences in bioavailability of Cu sources for goats.

3.3 Interactions influencing copper absorption

3.3.1 Introduction

Most interactions cause a lower availability of the element to the animals, thereby increasing the dietary concentrations necessary to cover the animal's requirement.
Experiments concerning the most important interacting dietary components reported in the scientific literature will be surveyed. These include the interactions of Cu with Mo, S, Zn, phytate and Fe. Interactions in cattle, sheep and goats were included in this search. As far as possible, effects are quantified. No data are available on any differences in interactions between dairy cattle and beef cattle.

3.3.2 Interactions of copper, molybdyenum and sulphur

3.3.2.1 Cattle

For a survey of the effects of Mo and S on Cu availability in cattle and sheep see reference [140].

3.3.2.1.1 Sources of dietary S

The main site for both Cu x S and the Cu x Mo x S interaction is the rumen [130]. Only degraded protein S and inorganic S (both yielding S^{2-}) are able to interact with Mo and Cu in the rumen. No information is available on differences between S from feed or drinking water. When reference values for sulphate and sulphide in drinking water are not exceeded, maximally about 15-20% (8.3-12.5 g/day[6]) of the daily S intake of cattle can be calculated to originate from drinking water [99]. In the ration, a maximum tolerable level of 4.0 g S/kg DM is suggested [98], although this limit is often exceeded in Dutch feeds (Table 2). High intakes of S entail the risk of polioencephalomalacia, which is characterized by neurologic symptoms: lethargy, anorexia, blindness, ataxia, sensory deficits of the skin of face and ears, muscle and ear twitches and eventually coma. Often the breath smells of H_2S [87;98].
The amount of S available for interaction with Cu absorption is influenced by the supply of rumen degradable protein and fermentable carbohydrates (variation in ruminal pH and S^{2-} absorption from the rumen), rate of eating ((dis)continuous supply of interacting components) and of S-compound degradation by rumen microbes. In the rumen, Cu can either bind to S, forming CuS (Cu^{2+}) and/or Cu_2S (Cu^{+}) or to Mo and S, forming thiomolybdates. Thiomolybdates contain 1-4 S-atoms per molecule (TM_1-TM_4). The S atoms are introduced in the thiomolybdate molecule at the expense of an O molecule. The composition of TM_1 is MoO_3S^{2-} and that of TM_4 is MoS_4^{2-}. Mainly TM_3 and TM_4 irreversibly bind Cu to high molecular weight proteins. Both Cu-S and TM's render the Cu unavailable to the animal, as Cu associated with these compounds is insoluble [130]. The effect of Cu-S compounds on Cu availability seems to be less important than that of thiomolybdates [117]. As Cu is probably mainly present as Cu^{+} in the rumen, Cu_2S will be the predominant Cu-S compound [94]. The more S is available in the rumen, the more "higher" thiomolybdates (TM_3 and TM_4) are formed, thus lowering Cu availability [130]. In sheep, no differences in plasma Cu concentrations were observed when comparing S from methionine and from Na_2SO_4, although S concentrations of the two groups were different (basal diet 1.0-1.3 g S/kg DM, supplied with either 1.7 (from $Na_2SO_4.10H_2O$) or 2.6 (from methionine) g S/kg of diet) [126]. However, as plasma Cu is not a very reliable indicator of Cu status [90], these results have limited value. On the other hand, trichloracetic acid (TCA) insoluble Cu in plasma (indicating the amount of absorbed TM's) is higher when elemental S instead of Na_2SO_4 is added to the ration (basal ration 2.2 g S/kg DM, supplied with 3 g S/kg DM from

[6] Calculated from: drinking water intake 100-150 L/day; current Dutch reference values of 250 mg/L (sulphate) and 0.02 mg/L (sulphide) [99]

either source) [79]. This indicates a higher amount of absorbed thiomolybdates and, hence, a greater reduction of Cu availability by the addition of elemental S when compared with Na_2SO_4.
Finally, at least TM_3 can be absorbed from the intestine and be detected in the portal blood. Systemic effects of TM's, e.g. due to the formation of a Cu-TM-albumin complex, cannot be excluded. However, it is not clear as to what extent these effects occur under normal physiological conditions [130].

3.3.2.1.2 Non-ruminating calves

In newborn calves, true Cu absorbability (A_{Cu}) can be up to 70%. Upon the development of the rumen (implying the development of a sulphide-producing flora), A_{Cu} decreases substantially [98]. In veal calves on a milk-substitute ration, proper ruminal development and function and, hence, production of S^{2-} and thiomolybdates is minimal. Therefore, the Cu x Mo x S interaction will be irrelevant for this type of animals. Besides a milk replacer, veal calves can receive extra maize silage (0.3-0.5 kg DM/day). Maize silage contains on average (DM) 1.0 g S/kg and 0.4 ppm Mo (Table 2). Using the equation for the calculation of A_{Cu} from grass silage [140], A_{Cu} from maize silage would be 10.6%, which is a relatively high value for roughage [140]. Therefore, although no information is available on the production of thiomolybdates on a milk-substitute / maize silage ration, in this case A_{Cu} is not likely to be seriously hampered by dietary Mo and S.

3.3.2.1.3 Ruminating animals

If grass products (e.g. grass, grass silage) are the major constituents of the diet of ruminants, the major determinants of Cu availability may be the Mo and S content of the feed. However, also in concentrate-type diets A_{Cu} is affected by both the Cu x S and Cu x Mo x S interactions. Thiomolybdates per se (without Cu) are unstable, but derive their stability from association with the solid phase in the rumen. Thiomolybdates containing 3 or 4 S-atoms ($MoOS_3^{2-}$ (TM_3) and MoS_4^{2-} (TM_4)) cause Cu to be irreversibly attached to high molecular weight proteins and thus reduce Cu absorption [130]. Possibly, differences in the solid phase and/or protein composition of rumen contents of animals consuming different types of roughage may contribute to the observed differences in A_{Cu} (see below).

3.3.2.1.4 Quantification of the Cu x Mo x S interaction

Underwood and Suttle [140] suggested the Cu x Mo x S interactions to be influenced by the nature of the roughage. To quantify Cu x Mo x S interactions, experiments were carried out using 28 hypocupraemic ewes fed fresh herbage (2.7-3.5 g S and 0.7-24.4 mg Mo/kg DM) or hay (3-3.4 g S and 0.4-18.7 mg Mo/kg DM) [129]. Responses in plasma Cu, as well as dilution of intravenously infused ^{64}Cu ("intravenous repletion technique") when feeding the several diets were used to assess Cu availability [125]. No suitable data are available on experiments with silages and / or the influence of dietary S. In grass silages S reduces A_{Cu} in a logarithmic manner [140]. A_{Cu} is calculated using the equation (S: g/kg DM):

$$A_{Cu}(\%) = 10.6 - 6.65 \ln S \qquad \text{(I)}$$

In hay, the inhibitory effect of Mo on Cu availability is detectable but less than that of S and is not greatly influenced by interactions with S. A_{Cu} remains relatively high. A_{Cu} is calculated using the equation (Mo: ppm (DM); S: g/kg DM):

$$A_{Cu}(\%) = 8.9 - 0.7 \ln Mo - 2.61 \ln S \qquad \text{(II)}$$

In fresh grass, A_{Cu} starts low and is further substantially reduced by small increments in Mo and S. A_{Cu} is calculated using the equation (Mo: ppm (DM); S: g/kg DM):

$$A_{Cu}(\%) = 5.7 – 1.3\ S – 2.785 \ln Mo + 0.227\ (Mo \times S)\ [140]. \qquad (III)$$

However, in a critical evaluation of the above equations Jongbloed et al [69] noticed that equation (I) is rather unphysiological as A_{Cu}=0 at dietary S levels > 5 g/kg DM. Moreover, equation (III) yields unreliable results as A_{Cu} would increase at dietary S levels > 3g/kg DM together with dietary Mo levels > 4 ppm (DM). Finally, there is neither convincing evidence (1) that A_{Cu} really differs between different roughages, (2) that the intravenous repletion technique has been applied throughout all experiments, (3) that the equation to calculate A_{Cu} resulting from the intravenous repletion technique has been validated under various dietary circumstances (varying Mo and S concentrations) nor (4) that results obtained in sheep are applicable in cattle. Therefore, both equations (I) and (III) have to be rejected for calculation of A_{Cu} in ruminant rations. Only the course of A_{Cu} as calculated with equation (II) seems to be physiological for use in sheep rations. The same remark is valid for the outcome of the equation of Suttle and McLauchlan found in sheep [135]:

$$^{10}\log (A_{Cu}) = -1.153 – 0.0019 \times Mo – 0.0755 \times S – 0.0131 \times Mo \times S$$

or

$$A_{Cu}\ (\%) = 100 \times 10^{(-1.153 – 0.0019 \times Mo – 0.0755 \times S – 0.0131 \times Mo \times S)} \qquad (IIIa)$$

in which Mo = ppm (DM) and S = g/kg DM.

The outcomes of the equations II and IIIa for dietary S levels of 1 and 5 g/kg DM are visualized in Figure 1. As equation (II) yields relatively high values (A_{Cu} = ± 4% at dietary DM concentrations as high as 4 g S/kg and 8 ppm Mo), equation IIIa is arbitrarily chosen to be the best assessment of estimating A_{Cu}.

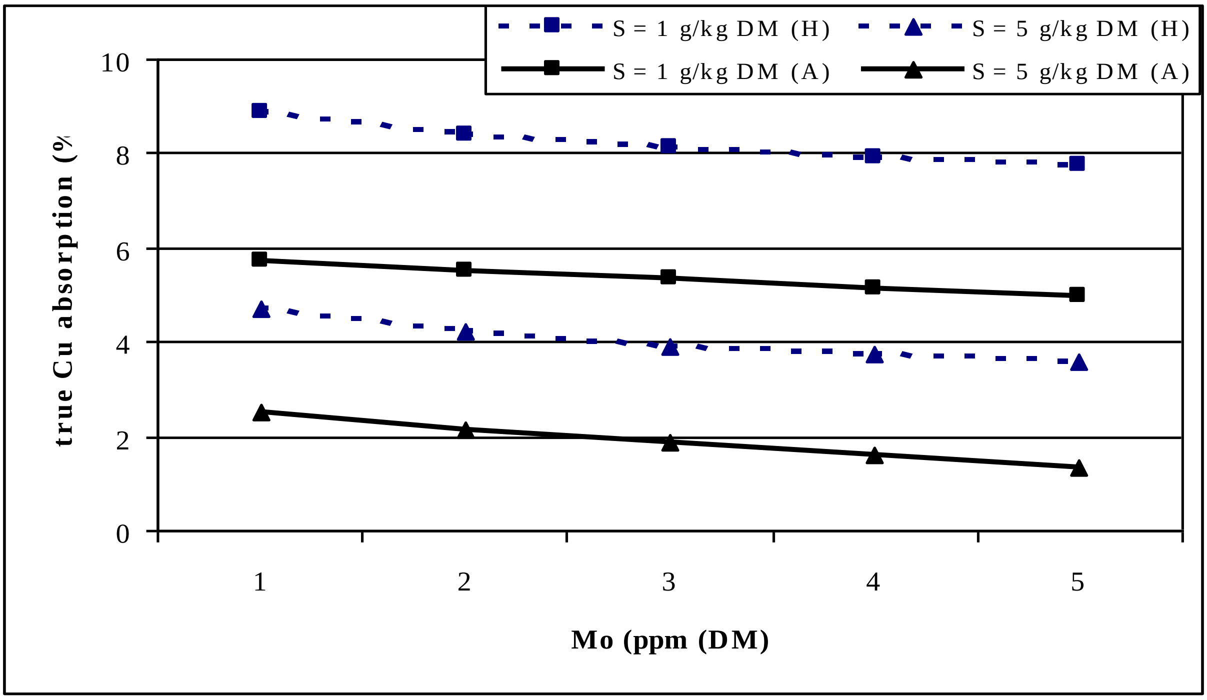

Figure 1 Comparison of two equations to calculate true Cu absorption (A_{Cu}) in sheep (H = equation for hay (II) [140] and A = equation for all roughages (IIIa) [135].

For practical use, usually the Cu:Mo ratio (ppm (DM)) in the feed is used to predict risks for Cu deficiency and toxicity. Although these ratios require flexible interpretation, ratios < 1 generally indicate a high risk, whereas ratios of 1-3 indicate a marginal risk of Cu deficiency [140] (see "Criteria to judge copper status").
In 2003, Dutch grass silages contained on average 2.8 g S/kg DM and 2.1 ppm Mo (DM). For grass silages harvested in 1999, detailed data are given in Table 2.

Table 2 Ranges (upper and lower limits) and mean trace mineral concentrations of Dutch grass silage harvested in 1999 (BLGG, Oosterbeek, The Netherlands, 2000)

	Grass silage from soil type:								
	Clay			**Sand**			**Peat**		
Trace mineral	Mean	Lower	Upper	Mean	Lower	Upper	Mean	Lower	Upper
Zn (ppm (DM))	34	20	62	48	30	74	40	25	55
Fe (ppm (DM))	356	115	950	311	120	800	538	110	1400
Cu (ppm (DM))	6.9	4.8	10.4	7.8	5.2	11.4	8.2	5.4	11.5
S (g/kg DM)	3.0	2.0	4.3	2.9	1.8	4.2	3.0	2.1	4.2
Mo (ppm (DM))	2.4	0.9	4.9	2.3	0.9	4.7	2.6	0.9	5.4

Maize silages contained on average 1.0 g S/kg DM, 0.4 ppm Mo and 3.8 ppm Cu (DM) (BLGG, Oosterbeek, The Netherlands, 2004).
For grass silage containing 2.8 g S/kg DM and 2.1 ppm Mo (DM), using equation (IIIa), A_{Cu} is approximately 3.7%.

3.3.2.2 *Cattle and sheep*

As most experiments have been carried out in sheep, the above equations regarding the effects of S and Mo on A_{Cu} are applicable to these animals. As the experiments have been carried out using Scottish Blackface sheep[7], the outcome is supposed to apply to "average" sheep with respect to Cu requirements. Sheep breeds sensitive to Cu deficiency may have lower A_{Cu}, whereas sheep sensitive to Cu toxicosis may have higher A_{Cu} values compared with the values calculated with equation IIIa. Unfortunately, it is not clear as to what extent these equations can be used for dairy cattle [140]. For beef cattle, an equation assessing required dietary Cu concentrations using whole ration Mo and S concentrations has been developed (see 4.1). As no more suitable data are available, this equation is also used for dairy cattle. Differences between dairy and beef cattle to be accounted for with regard to Cu absorption may be:

- differences in ruminal transit time of feed (related to DMI),
- differences in ruminal pH and degradability of feed components,
- differences in ruminal concentrations of Cu, Mo and S,
- age effects.

3.3.2.3 *Goats*

Hardly anything is known concerning the Cu x Mo x S interaction in goats [95]. Therefore, it is assumed that the interaction resembles that in sheep.

3.3.2.4 Examples of A_{Cu} from several rations

In Table 3, some estimates are made for true Cu absorption (A_{Cu}) from examples of bovine and caprine rations based on Mo and S concentrations. For fresh grass, hay and grass silages mineral values of BLGG (Table 2) and for straw mineral values of NRC [98] are used. Equation IIIa is used to calculate A_{Cu}. As maize silage contains lower concentrations of S

[7] Scottish Blackface sheep are not very prone to Cu toxicosis (see Chapter 7).

and Mo, A_{Cu} from maize silage is higher than that from grass silage. Using the actual Mo and S concentrations in Dutch maize silage and using equation IIIa A_{Cu} can be calculated to be 5.9%. For concentrates, a value of 9.1% as given for cereals (Table 11.1 in ref. [140]) is used[8]. Predictions of A_{Cu} from roughage / concentrate rations are speculative [140]. This may be even more valid for grass silage/maize silage/concentrate rations and for extra maize silage feeding to grazing animals. In the latter case, lack of data on DMI from fresh grass even precludes any calculation of A_{Cu}. Here, it is assumed that, if DMI of the different ration components is known, A_{Cu} of the total ration can be calculated as the weighted average of those of the ration constituents.

Table 3 Examples of estimates of A_{Cu} from ruminant rations

Category	**Ration (kg DM)**	**Estimated A_{Cu} (%)**	
		Separate	Mean
Cow, dry, 40 weeks pregnant	grass silage 7.5 barley straw 1.5	3.6 5.5	3.9
Cow, lactating, 40 kg milk	fresh grass 12.9 concentrate 9.0	3.6 9.1	5.8
Sheep/goat, dry, last term pregnant	hay 1.0 concentrate 0.5	3.6 9.1	5.4
Lamb, growing	fresh grass	3.6	3.6
Goat, lactating, 5 kg milk	hay 1.0 concentrate 2.3	3.6 9.1	7.4

3.3.3 Interactions of copper and iron

3.3.3.1 *Cattle*

Antagonism between Cu and Fe can also affect (A_{Cu}). To roughly assess the effect of Fe on Cu absorbability, Fe:Cu ratios can be used [140]. Ratios > 100 indicate a high risk, whereas ratios of 50 to 100 indicate a marginal risk of past or future Cu deficiency.

Calves on a milk-substitute ration containing either 10, 40, or 100 ppm Fe (DM) and either 0.5 or 5.5 ppm Cu had similar blood Cu, caeruloplasmin and hepatic Cu concentrations [19]. The same investigators [20;58] found no effect of the addition of 50, 250, or 500 ppm Fe on liver Cu retention in preruminant Friesian calves on a milk-substitute ration (2 ppm Cu (DM)). After weaning, liver Cu concentrations declined when additional Fe was given. Selected results of this trial, as well as results of others, are given in Table 4. Normal milk replacers contain 45 ppm Fe (first 6 weeks of fattening period) to 8-10 ppm Fe (7-23 weeks) [147] or 2.3 - 92 ppm Fe [9] [142] in order to avoid a red meat colour. As these concentrations on average are much lower than those used in the experiments described above, impairment of Cu absorption by high dietary Fe concentrations will, therefore, be of no practical significance in veal calves. On the other hand, the very low Fe concentrations in milk replacers are thought to play a role in the extreme high accumulation of Cu in the liver of veal calves [47].

The addition of ferrous sulphate to the ration of steers depressed the apparent Cu absorption and also lowered hepatic Cu concentrations [122]. Similar observations were made in dairy cows [25]. However, the effects produced by ferrous sulphate could be theoretically related to the formation of sulphide and / or thiomolybdates in the rumen and thus be independent of iron. As an example, the amount of Fe (500 mg/kg DM) from $FeSO_4$ used in dairy cows [25] implied the addition of ± 0.3 g S/kg DM to a ration containing 2.5 g S/kg DM. The S addition itself can, therefore, be assessed to be responsible for a reduction

8 No data on Cu and S concentrations given. Mo content is supposed to be < 2 ppm (DM).

9 No data available on category or stage of fattening period (starting, finishing etc.)

of A_{Cu} of ± 0.8%. Results of these experiments have, therefore, not been included in this study.

In several experiments, differences in clinical appearance between Fe- and Mo-supplemented animals were remarkable (for details see Table 4) [20]. Although both groups were Cu-deficient (low liver Cu concentrations), the Mo-supplemented animals showed typical signs of Cu deficiency (changes in hair texture, coat depigmentation, stilted gait), whereas the Fe-supplemented animals did not. Feed intake, weight gain and feed efficiency were lower in the Mo-treated group than in the Fe-treated and untreated groups. Moreover, Mo-treated animals showed higher liver Fe and lower plasma Fe concentrations, particularly when growth rate was affected [104]. The reason for these differences between Fe- and Mo-treated animals is not clear. The reduced weight gain could be explained by the impaired feed intake and feed efficiency. The coat changes have been suggested to originate from selective accumulation of Mo in the skin, producing a local Cu deficiency. On the other hand, gait changes could be explained from increased Mo rather than decreased Cu concentrations in (connective) tissues. Molybdenum can be absorbed as a thiomolybdate (3.2.1.1). Release of Mo from this compound is uncertain, and under such circumstances lower activities of Mo-dependent enzymes (e.g. xanthine oxidoreductase [124]) are possible. Stiffness of gait might owe to conversion of xanthine oxidoreductase (from dehydrogenase (D) to oxidase (O) type) which is thought to be involved in gait changes in rheumatoid arthritis. Gait changes are frequently seen in cattle grazing Mo-rich pastures, whereas sheep under similar conditions are not affected. As bovine tissues contain higher levels of the type O activity of xanthine oxidoreductase than do sheep tissues, this finding could corroborate the hypothesis [130].

Table 4 Selected results from experiments on the influence of dietary Fe supplements on Cu status (n = number of animals; wk = weeks on experiment)

				Total dietary content (DM)				**Liver Cu**		**Plasma Cu**		
Ref	**Category**	**n**	**wk**	**Cu**	**Fe**	**Mo**	**S**	**Initial**	**Final**	**Initial**	**Final**	**Type of ration**
					ppm		g/kg	ppm (DM)		µM		
[60]	beef calves	5	32	4.7	100	0.1	2.8	110	72	12.3	15.7	barley grain + barley straw; supposed to contain 85% DM
		5			941	0.1		95	4	11.8	3.3	
		5			100	6.0		105	2	12.3	2.4	
		5			941	6.0		104	2	12.9	2.2	
[104]		7	32	4.0	100	0.1		129	53	15.9	11.5	
		7			600	0.1		134	6	15.1	2.7	
		14			100	5.1		125	4.0	15.6	1.9	
[20]		6	24	4.0	100	0.1			43		9.3	
		6			396	0.1			9		6.8	
		6			693	0.1			13		3.0	
		6			989	0.1			7		3.0	
		5	41		100	0.1			95		10.9	
		5			278	0.1			10		8.4	
		5			100	2.4			3		1.5	
		5			278	2.4			1		0.9	
[40; 41]	beef cows	9	40	4.5	204	1.5	3.0			9.4	12.8	maize silage / soybean meal / urea
		10			804	1.5				11.4	12.9	
		10			204	6.5				13.2	4.3	
	matching calves	5	± 26		204	1.5				11.3	3.0	
		8			804	1.5				11.5	1.9	
		9			204	6.5				11.7	1.1	
[45a]	grazing lambs	6	12	7.8	206	0.13	3.9		239			ryegrass / white clover pasture
		6			689 [a]				131			

[a] extra Fe supplied orally as $FeSO_4.7H_2O$ in gelatin capsules

As can be seen in Table 4, liver Cu concentrations are mainly influenced by dietary Fe concentrations when Mo concentrations are very low (i.e. 0.1 ppm (DM)). Due to lack of data it is difficult to determine the Mo concentration at which the Fe effect on Cu metabolism is

overruled by the Mo effect. However, at 1.5-2.4 ppm Mo (DM) some Fe effect can be observed, whereas no Fe effect is detectable when Mo concentrations are 6.0 ppm (DM). This separate Fe-effect can be explained by the fact that Fe interferes with Cu absorption in another way as Mo does. Iron is suggested to form FeS in the rumen. Subsequently, in the abomasum the S is released from FeS to form CuS. This hampers Cu uptake, which does not start anterior to the small intestine [62]. Thus, the action of Fe on A_{Cu} is independent from, but not really additive to the thiomolybdate action. Besides this, Fe_2O_3 can adsorb Cu and in this way reduce A_{Cu} [130]. When dietary Mo concentrations increase (e.g. to 2.4 ppm (DM)), the Mo concentrations fully determine the liver Cu contents, without any additional effects of dietary Fe. This is in accordance with the findings of others [140].
The possible antagonistic effect of dietary Fe on Cu absorption may be of practical importance, as the Fe content of silage and forage, including dust or rain soil splash, can range from 50 to 4000 ppm (DM) [60], and levels within this range could potentially influence Cu absorption in ruminants [113]. The levels mentioned in Table 4 (e.g. 800 ppm additional Fe) are thought to represent the amount of Fe in silage "available" for interaction with Cu absorption along the intestine [60]. Soil can also hamper Cu absorption in ruminants [132; 133], but as soils contain varying amounts of Fe and Mo (and Cd and Zn, see 3.2.3 and 3.2.5) [131], the effect of soil must be interrelated with those of the trace elements mentioned. Moreover, differences in impairment of Cu absorption exist between soil types (maybe due to differences in physical adsorption of one or more of the interacting components to the soil solids), clay and chalk soils causing a lower A_{Cu} (1.7%) than does sand (3.0%)[10] [131; 132]. The inhibitory effect of ingested soil on Cu metabolism has been shown to be dependent on the dietary S content. Chalk and clay soils containing 1500-2800 ppm Fe (DM) reduced plasma Cu concentrations when the S content of the diet was 4.1, but not when it was 1.0 g/kg DM [131]. Iron was suggested to be the most likely soil component to interact with S, as only soils low in Fe did not affect Cu metabolism. Due to the different levels of Cd, Zn and Mo in the different soil types used, assessing the availability of Fe from soils compared with that from $FeSO_4$ is difficult. However, adding 800 mg Fe (DM) from $FeSO_4$ to a semi-purified diet of Cu-depleted ewes resulted in a similar true Cu absorption (1.5%) as the clay and chalk soils (1.7%; 2800 and 1500 ppm additional Fe (DM), respectively)[132]. The non Fe-supplemented control group and the sand-supplemented group (140 ppm additional Fe (DM)) had substantially higher absorption values (3.1 and 3.0%, respectively). Finally, soil intake will vary depending on weather and soil type and sod quality.
In conclusion, assuming the Fe x Cu interaction is the same in rations containing insufficient and sufficient Cu, in rations of very low Mo content (0.1 ppm (DM)), from the data presented in Table 4 the relation between dietary Fe concentration and either plasma or liver Cu concentrations (24-32 weeks experimental period) can be roughly calculated by the equations:

$$\text{plasma Cu } (\mu M) = 218.26 \times Fe^{-0.628} \qquad R^2 = 0.88$$

$$\text{liver Cu (ppm (DM)} = 8155.3 \times Fe^{-1.0812} \qquad R^2 = 0.87$$

in which Fe = Fe content of the ration (ppm (DM)).

No suitable data on the bioavailability of Fe in drinking water on Cu absorption in cattle are available. As an example, the quantitative significance of the amount of Fe from drinking water can be estimated. The Dutch maximum tolerable level of Fe in drinking water for cattle is 2.5 mg/L [27]. If this threshold value is not exceeded, Fe intake from drinking water of a dairy cow consuming 120 L water/day will be up to 300 mg Fe/day. Average Fe content (all Dutch soils) of grass silages is approx. 400 ppm (DM). Lactating dairy cows consuming a

10 Absorption coefficients for Cu calculated from the increments in plasma Cu relative to that produced by intravenously applied Cu [125]

ration of 11.9 kg DM of grass silage (400 ppm (DM)) and 12 kg of concentrates (200 ppm Fe) have a total daily Fe-intake of 7160 mg. Similarly, on a ration consisting of 11.9 kg DM of maize silage (200 ppm Fe (DM)), 4 kg of soybean meal (230 ppm Fe) and 8 kg of concentrates (200 ppm Fe) daily Fe-intake will be 7280 mg. The highest Fe concentration in ground and surface water in the Netherlands is ± 8 mg/L. Even then, Fe intake from drinking water will not exceed ± 1000 mg/day. Under normal conditions, the contribution of Fe from drinking water will, therefore, be relatively minor. As discussed in CVB Documentation Report Nr. 40 (CVB, 2005), the contribution of elemental Fe from wastage of concentrate processing equipment can be ignored.

3.3.3.2 Sheep

In two experiments with sheep [3; 48] the effect of Fe from $FeSO_4.7H_2O$ on Cu metabolism was measured. Concerning the use of $FeSO_4$ the same remark as made for the cows is valid. However, as the additional amount of Fe used in one of the experiments was small (15 ppm Fe [3]), the extra amount of S supplied in this way was very small (8 mg/kg of diet). The results of this experiment were included in this study, whereas those from the other trial [48] were not.
Selected results of two suitable experiments are given in Table 5.

Table 5 Selected results from two experiments on the influence of Fe on Cu metabolism (n= number of animals/group; wk = weeks on experiment)

			Total dietary content (DM)					**plasma Cu**		
Ref.	**n**	**wk**	**Cu**	**Fe**	**Mo**	**S**	**Final liver Cu**	**initial**	**final**	**type of ration**
			ppm			g/kg	ppm (DM)	µM		
[3]	4	12	4.7	22	0.75	1.8	198	7.8	7.8	maize / rice bran / groundnut cake / palm kernel meal / forage[b]
	4			39			196	7.8	6.3	
	4		10.3[a]	22			822	7.8	15.7	
	4			39			769	7.8	15.7	
[105]	6	±17	4.0	169	1.2	2.9	297	14.0	14.3	grass pellets / maize[c]
	6			499			270	14.8	13.1	
	6			829			242	14.3	11.5	
	6			1488			186	14.6	10.2	

[a] extra Cu from $CuSO_4.5H_2O$; [b] supposed to contain 88% DM; [c] supposed to contain 91% DM.

Due to scarcity of data comparison with cattle data (Table 4), quantification of the Fe-effect is difficult. In general, duration of the cattle experiments was longer, whereas hepatic Cu concentrations were lower. On the other hand, the 1488 ppm Fe (DM) concentration in experiment [105] substantially exceeds the concentrations used in cattle. The differences in total dietary Fe concentrations in experiment [3] seemed to be too small to evoke clear alterations in Cu status. However, also in sheep Fe reduces Cu status.
The presence of soil in roughages can also hamper Cu absorption in sheep. True Cu absorption[11] in sheep consuming a roughage-based ration containing 10% soil (DM basis) was lowered by ± 50% (from 3.1 to 1.7% [132;133]). This research group demonstrated clay and chalk soils (supplying 1500-2800 ppm Fe (DM)) to lower plasma Cu concentrations in sheep [132]. After 21 days on the experimental diet (9.9 ppm Cu, 4 g S/kg (DM), Mo content not given) plasma Cu concentrations were lower (no statistical data given) when compared to a control group not ingesting soil. In a second experiment this effect could not be repeated, whereas a Fe supplement (supplying 800 mg Fe/kg diet DM from $FeSO_4$) was equally effective in both experiments. Factors other than Fe in soil may, therefore, affect Cu absorption.

[11] Absorption coefficients for Cu calculated from the increments in plasma Cu relative to that produced by intravenously applied Cu [125].

3.3.3.3 Goats

In Dutch White dairy goats (51.0 kg BW, 6 animals) on a hay/concentrate ration, Schonewille et al. [113] found significant reductions in plasma Cu, caeruloplasmin activity and hepatic Cu concentrations when a high dose of Fe was added to the ration. The experimental rations contained either 269 or 2380 mg Fe/kg DM (from ferrous fumarate; 56x56-day cross-over design). After feeding the high-Fe instead of the low-Fe ration, plasma Cu concentrations were 14.4 vs. 17.6 µM, plasma caeruloplasmin activities were 273 vs. 315 absorbance units, whereas hepatic Cu concentrations were 2.9 vs. 4.0 µg/g DM, respectively. However, only hepatic Cu concentrations were significantly lower in the high-Fe compared to the low-Fe group ($p = 0.0497$).

3.3.4 Interactions of copper and zinc

Interactions between Cu and Zn within the intestinal tract are important for the amount absorbed of these elements. In rats, mainly increased concentrations of Zn can induce the synthesis of thionein. This protein binds Zn or Cu, thereby forming metallothionein [29]. As Cu is bound to thionein in intestinal cells, it is less available to the animal. By desquamation of the intestinal cells Cu is subsequently lost with the feces. Therefore, excess dietary Zn may cause a shortage of Cu. In sheep, this induction of metallothionein formation is far less than in rats. Dietary concentrations up to 48 ppm Cu and up to 543 ppm Zn do not or hardly induce intestinal metallothionein formation [110]. Therefore, it is not clear as to what extent Zn-induced metallothionein formation can influence Cu absorption from the intestine in ruminants.

3.3.4.1 Cattle

No data on a Cu x Zn interaction in cattle are available.

3.3.4.2 Sheep

In sheep, a protective effect against Cu toxicosis of feeding either 220 or 420 ppm Zn compared with 43 ppm Zn in lambs was observed [21][12]. However, these Zn concentrations are extremely exceeding those found in normal Dutch silages (Table 2) and are, therefore, in general of no practical value. However, in the vicinity of Zn-processing industries values up to 601 ppm Zn (DM) have been found in maize silage [83]. In such areas the Zn content of the forages will be high enough to hamper Cu absorption.

3.3.4.3 Goats

No information is available on any interaction of dietary Cu and Zn in goats.

3.3.4.4 Conclusion

In Dutch roughages, the Cu content is reported to be 4.8-10.4 ppm (DM) and the Zn content is 20-62 ppm (DM) (Table 2). As discussed by Jongbloed et al. [68], for Dutch cattle current practical levels in concentrates are 27-32 ppm Cu (DM) and 78-85 ppm Zn (DM). As there is no direct evidence, it is not possible to judge the importance of Cu*Zn interactions in ruminants. However, in the light of the values mentioned for sheep compared with the current practical Dutch levels, Cu-Zn antagonism does not seem to be of practical

[12] Assuming a DM content of the barley/fishmeal/distillers dark grains ration of 90%, these concentrations correspond with 244, 467 and 48 ppm Zn.

importance for ruminants [140], except ruminants receiving forage harvested in the vicinity of Zn-processing plants.

3.3.5 Interactions of copper and phytate

Phytate can affect the bioavailability of several minerals and trace elements [72]. Xu et al. [156] carried out an experiment with two groups of veal calves. The control group was fed milk replacer as the sole feed. In the ration of the experimental group, part of skim-milk powder in the milk replacer was replaced by soybean protein concentrate. When compared to the control group, in the experimental group no effect on hepatic Cu concentrations was observed, although plasma Cu concentrations in this group were slightly lower during the first weeks of the experiment.
As in ruminants the ruminal flora produces phytase, no negative effect of phytate on Cu or Zn bioavailability in ruminants may occur. As yet, no experimental results on this subject are available (H. Valk, personal communication).

3.3.6 Interactions of copper and cadmium

3.3.6.1 Cattle

In fattening bulls, Anke et al. [10] could not demonstrate significant effects of the addition of 3 ppm Cd on liver Cu concentrations. When supplements of either 10 g S/kg and 10 g S/kg + 3 ppm Cd to a ration (no further details given) were given for 28 weeks, liver Cu levels were 36 and 46 ppm (DM), respectively.

3.3.6.2 Sheep

No data are available on any effect of Cd on Cu metabolism in sheep.

3.3.6.3 Goats

In two reports concerning pregnant goats supplemented with 75 ppm Cd (semi-synthetic diet, no data available), reductions of Cu contents of tissues of both dams and kids were demonstrated [8;9]. None of the lambs born from dams receiving 75 ppm Cd was viable, whereas 50% of the dams in this group aborted. Selected data are given in Table 6. In the second experiment [8], Cu concentrations in liver, kidneys and hair were very similar to those reported for the first experiment. Possibly the data of the second report originated from the same experiment. No statistical data were given.

3.3.6.4 Conclusion

Under practical circumstances, Cd concentrations in herbage from non-polluted areas range from 0.1-0.8 ppm (DM), whereas those in industrially polluted areas is 1-21 ppm (DM) [91]. The results obtained with bulls and sheep are, therefore, more or less of practical importance. The reported goat data can be ignored because the Cd levels applied substantially exceed those occurring in practice.
Regarding the relatively low Cd level applied, the Cd effect on Cu status (if any) in the experiment with bulls may have been overridden by the relatively large S addition. In ewes and lambs, Cd levels similar to those occurring in practice may depress Cu levels in blood and tissues. However, as Cd effects are less evident in mature sheep than in lambs and the relative decreases in liver Cu concentrations observed are rather different between the experiments, quantification of the Cd effect on Cu status is precluded.

Table 6 Selected data of Cu status from experiments with sheep and goats given graded dietary Cd supplements

Ref	Category	n	Total Cd (ppm)	Blood Cu (µM)	CP (IU/mL)	Cu concentration (ppm (DM))				
						ppm (DM)			ppm	
						Liver	Spleen	Kidney	Milk	Hair
[91]	Ewes	4	0.7			185				
		4	3.5			174				
		4	7.1			131				
		4	12.3			59				
	matching lambs	5	0.7	13.2	27	101				
		5	3.5	8.8	18	22				
		4	7.1	9.9	22	62				
		6	12.3	6.9	15	13				
[32]	Lambs	6	0.2			688	12.70	14.9		
		6	5.2			318 D	7.98 D	18.6		
		6	15.2			363 D	7.72 D	17.6		
		6	30.2			290 D	6.90 D	18.8 D		
		6	60.2			194 D	8.22 D	20.0 D		
[80]	Wethers	?	0	12.9		123				
			7.5	11.3		83				
			15	10.1 D		75 D				
[9]	female goats	?	0 (a)			39 (b)		47 (b)	2.7	8.9
			75 (a)			16 D,b		40 (b)	1.4 D	2.7 D
	matching kids		0 (a)			218 (b)		90 (b)		8.3
			75 (a)			19 D,b		26 (b)		9.4

CP = Caeruloplasmin activity; D = significantly different from lowest Cd level; (a) = added Cd; (b) = assuming DM contents of liver (29%) and kidney (21%) [14]; n = number of animals[89].

3.3.7 Interactions of copper and either calcium and/or phosphorus

3.3.7.1 Cattle

When a maize silage/hay/concentrate ration of lactating cows (mean milk yield 17.5 kg) was enriched in Ca, the course of liver Cu concentrations resembled that of cows on the non-supplemented ration [57]. When both Ca and P were increased, the decrease in liver Cu content was significantly less than in the other groups. The non-supplemented ration contained 8.8 g Ca, 4.7 g P and 6 mg Cu/kg. In the Ca-supplemented ration (from $CaCO_3$) the Ca content was increased to 23.0 g Ca/kg, whereas the Ca and P supplemented ration (from tricalcium phosphate) contained 23.0 g Ca and 12 g P/kg. The experiment lasted for 12 months. Initial liver Cu concentrations were approx. 200 ppm (DM). Final liver Cu concentrations were 58 (non-supplemented), 77 (Ca-group) and 134 ppm (DM)(Ca + P group). The reason for this difference is not clear.

In steer calves, the addition of P (from NaH_2PO_4) and Ca (from limestone, 38% Ca) to a maize/hay/soybean meal ration slightly depressed liver and spleen Cu concentrations and apparent Cu absorption [121]. The low- and high P rations contained 2.3 g P and 2.6 g Ca/kg vs. 4.6 g P and 5.2 g Ca, respectively. After 77 days, liver Cu concentrations were 134 and 93 ppm (DM) for low- and high P rations, respectively.

3.3.7.2 Sheep

No data are available on any effect of dietary Ca and/or P on Cu metabolism in sheep.

3.3.7.3 Goats

In non-lactating goats (56 kg BW), feeding a barley straw/concentrates ration containing either 3.8 or 15.4 g Ca/kg (from $CaCO_3$) for 28 days, had no significant effect on apparent Cu absorption or plasma Cu concentrations [112].

3.3.7.4 Conclusion

In the few experiments mentioned unphysiologically high Ca and/or P concentrations were employed. Therefore, although an increase of both Ca and P within physiological limits tends to depress liver Cu concentrations and apparent Cu absorption in calves, no convincing evidence has been reported as to any practical importance of increased dietary Ca and P concentrations. Increasing solely the dietary Ca concentrations does not influence Cu metabolism in ruminants.

3.3.8 Interactions of copper and lead

3.3.8.1 Sheep

The addition of up to 1000 ppm Pb during 75-84 days to a ration consisting of maize, cottonseed hulls, soybean meal and grass hay fed to wethers did not significantly influence Cu concentrations in tissues [37; 102].

3.3.8.2 Cattle and goats

No data are available on the interaction of Pb and Cu in cattle or goats.

3.3.8.3 Conclusion

Practical levels of Pb on forage downstream a lead smelter are reported to be 163-212 ppm compared with 5 ppm before the start of the smelter [35]. Assuming a DM content of fresh grass of 16% [24], these concentrations are 1019-1325 and 31 ppm (DM), respectively. This value is in accordance with others (500-1000 ppm (DM)) in Pb-contaminated areas. Lead-dust contaminated plants cannot be easily cleaned with water [50]. The Pb concentrations used in the sheep experiment are, therefore, applicable to Pb-polluted areas. However, as even at these Pb levels no effect of Pb on tissue Cu accumulation could be demonstrated, Pb x Cu interactions are of no practical importance.

3.3.9 Interactions of copper and manganese

3.3.9.1 Sheep

Sheep fed 8000 ppm additional Mn from MnO for 6 weeks showed significant increases of serum and renal Cu concentrations, whereas those in liver and muscle were slightly increased [18].

3.3.9.2 Cattle and goats

No data are available on any interaction of Mn and Cu in cattle or goats.

3.3.9.3 *Conclusion*

As the Mn level used in the sheep experiment extremely exceeded that found in forages (e.g. sweet clover (20 ppm (DM)) [38], these results do not supply convincing evidence as to any Cu x Mn interaction under practical circumstances.

3.4 Recycling

Part of the Cu that is excreted via the bile into the intestines, as well as Cu from desquamated intestinal cells is subsequently taken up (enterohepatic recycling). The extent to which recycling occurs in ruminants, and its vulnerability to impairment by thiomolybdates, remains to be investigated [140]. However, studies with wethers on semi-purified diets (10.8 ppm Cu) showed a 2.5–fold increase in faecal endogenous Cu losses[13] after supplementation of the diet with 25 ppm Mo and 2.5 ppm S [119]. Comparing two diets (hay, barley, canola meal) containing either 5 or 40 ppm Cu (DM) fed to heifers during 2 months, biliary Cu excretion[14] was significantly higher (0.19 vs. 0.13 mg/6 h) in the 40 ppm-group [45].

3.5 Excretion

Excretion of Cu mainly occurs via the faeces. In all ruminants, the endogenous losses via the urine are constant [11; 140] and relatively low. They were estimated to be 0.05 mg/day in sheep and 0.2-0.5 mg/day in cattle. These values are probably estimated in adult animals [11]. In growing, weaned ruminants, urinary Cu excretion accounted for 1.8 (calves) or 0.7% (goat kids) of total daily Cu intake, whereas the contribution of faecal Cu was 57.5 vs. 60.5% of daily intake for calves and goats, respectively [70]. In wethers, the addition of 4 g S/kg and 50 ppm Mo to a ration (corn products and soybean protein, 13 ppm Cu, 0,3 g S/kg) for 120-180 days increased urinary excretion of Cu. Day-to-day variation in urinary Cu excretion was considerable [86]. The chemical nature of these Cu compounds in the urine is unclear. In the control group not receiving extra S and Mo urinary Cu output remained at a constant, low level. Anyhow, Cu excretion via the urine seems not to be very important in the regulation of Cu metabolism.

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[13] Measured using the fecal excretion of ^{64}Cu excretion after an intraruminal dose (via a fistula) of ^{64}Cu

[14] Measured using animals with a surgically modified duodenum enabling bile flow and -composition determinations [136]

4 COPPER REQUIREMENTS

The Cu requirements of adult animals are determined by the endogenous (inevitable) losses, secretion into milk and wool or hair growth. In growing and pregnant animals Cu is also deposited in growing (foetal) tissues. The total of these requirements for a certain animal category (e.g. adult, lactating sheep) is the net requirement. The gross requirement is defined as the net requirement x 100/A_{Cu} (with A_{Cu} in % units).

4.1 Cattle

4.1.1 Dairy cattle

Based on 4 experiments with a total of 9 groups of cows the ARC assesses the total endogenous Cu loss to be 7.1 µg/kg BW/day (number of animals unknown) [11]. One of the experiments is carried out using a semi-synthetic diet based on starch and urea. Using this value, for a 650-kg cow the endogenous loss is 4.6 mg/day. However, from an intravenous repletion trial with growing cattle (see below) Suttle derived a value of 3.4 µg/kg BW as maintenance requirement [127], rounded up to 4 µg/kg BW for cattle and sheep [140]. The Cu concentration in growing tissues (when the liver is included as part of the carcass) is 1.15 mg/kg [98]. In an intravenous repletion trial, 0.5 mg Cu/kg gain was calculated [127]. In order to calculate minimal requirements, the latter value is adopted for all ruminants.
The intravenous repletion trial mentioned above [127] was carried out using animals that were at the lower limit of plasma (7.9 µM) and liver (10-20 ppm (DM)) Cu concentrations and animals were intravenously repleted as to prevent hypocupraemia. The values obtained for both endogenous loss (maintenance) and growth can, therefore, be considered to be “minimal”. However, as the experimental evidence for the former value (7.1 µg/kg BW) seems to be more comprehensive, this value is used to calculate requirements.
In the pregnant uterus, an amount of 0.61 (140 days) to 2.07 mg/day (281 days) is incorporated [54], which can be simplified to 0.5 (<100 days), 1.5 (100-225 days) or 2.0 mg/day (>225 days) [98;98]. As no different Cu levels have been applied during gestation (dietary Cu level slightly increased from 10 (late lactation) to 12 ppm (DM) (late dry period) [54], qualification of the calculated values as “minimal” or “optimal” is precluded.
Colostrum is reported to contain 0.6 mg/kg [98]. In literature, different values are given for the Cu content of mature bovine milk (mg/kg milk; 1L of milk is supposed to be 1 kg): 0.02 [82], 0.03-0.17 [66], 0.05-0.30 [75], 0.07 [4], 0.1 [11], 0.11 [76], 0.12 [81], 0.13 [36], 0.15 [98], 0.16 [114] and 0.19 [120]. The National Research Council [98] has even recently increased the estimation from 0.10 to 0.15 mg/kg. However, the Nederlands Instituut voor Zuivelonderzoek (NIZO Food Research) uses a value of 0.02-0.05 mg/kg. This value is based on own research and data from the International Dairy Federation. The Cu content of milk is often overestimated due to easily occurring Cu contamination from the environment (G. Ellen, personal communication). The mean of these values (0.04 mg/kg) is adopted to calculate requirements. A substantial increase of the Cu content of the feed during 6 weeks did not result in an increased Cu concentration of the milk. Control cows received ± 300 mg/day via the feed, whereas cows in the experimental groups (6-13 animals) received 550-840 mg/day. Mean Cu concentrations in milk of control and experimental groups were 0.16 and 0.17 mg/kg, respectively [114; 115]. On the other hand, two experiments with cows consuming Cu-deficient hay/concentrate rations revealed substantial increases in milk Cu concentrations after feeding rations with high Mo concentrations. Some selected data are given in Table 7. No data on S- and Mo-contents of the basal rations were given. After feeding 50 ppm extra Mo (from $Na_2MoO_4.2H_2O$) during 225 days the Cu concentration of the milk was 0.43 ppm, while the Cu concentration of the milk from the control group (no Mo added) was 0.16 ppm [141]. However, if 5 ppm Mo was added to the ration, the Cu content

of the milk approximated that of the 50 ppm Mo-group (Table 7). The Mo treatment used in this experiment, therefore, did not consistently influence the Cu content of the milk. After 300 experimental days, all milk Cu concentrations were 0.06 or lower. When (in a subsequent trial) beside Mo also 3 g S/kg was added, Cu concentrations of the milk decreased. However, Cu concentrations of the milk substantially varied. If animals only received extra Mo, no clinical signs of Cu deficiency were observed. Rations containing 3 g added S/kg and at least 50 ppm Mo resulted in signs as alopecia and discolouring of the coat.
In another experiment [56], the animals were kept on a diet containing 53 ppm Mo for 2 months. Subsequently, half of the animals were placed on a ration containing as much as 173 ppm Mo. Initial mean Cu concentration of the milk was 0.10 ppm. Final milk Cu concentrations were substantially higher (Table 7). Some animals receiving 173 ppm Mo showed growth retardation relative to those receiving 53 ppm Mo.
In itself, milk Cu concentrations could be expected to decrease after addition of Mo and/or S to the ration. The formation of thiomolybdates reduces Cu uptake from the intestines, thereby reducing the amount of Cu available for secretion into milk. Neither the reason for the increase in Cu contents of the milk observed in these experiments, nor the exact nature of the Cu compound determined in the milk is clear.
Most of the data from these two experiments cannot be used to reliably assess the milk Cu content of Dutch dairy cows, as the (added) Mo concentrations used far exceed those usually present in Dutch forages (Table 2). Moreover, dietary composition is not sufficiently described (S- and Mo concentrations of the basal rations), thus precluding sound interpretation of the data.

Table 7 Some selected data of 2 trials with dairy cows on diets with several amounts of added Mo and/or S (n = number of animals per experimental group)

Trial	Length (days)	N	Dietary Cu (ppm)	Dietary Mo (ppm)	Added S (g/kg)	Cu milk (ppm)	Cu serum / blood (μM)	Cu liver (ppm (DM))
[141]	225	3	2	0 (added)	0	0.16	12.6	122
		3		5 (added)	0	0.41	11.8	77
		3		0 (added)	3	0.03	11.5	85
		3		50 (added)	0	0.43	10.7	18
		3			3	0.03	11.0	14
[56]	120	4	6.4 (DM)	53 (total)	0	1.11	17.5	25.1
		4		173 (total)	0	1.31	18.6	12.6

Contrary to the NRC [98], it seems to be more reliable to adopt a value of 0.04 mg Cu/kg milk.
It can be concluded that mainly the animals not receiving concentrates (growing and dry cattle) can be prone to the development of Cu deficiency. This effect is most extreme on roughages from peat and clay soils, as the Cu requirement on such rations is relatively high. In a report on the Cu status of Dutch dairy cattle [100], the Cu status of dairy cattle grazing peat soils in the western part of the Netherlands was surveyed. Both Cu, Mo and S intakes of cattle in this area are high, whereas grass (silage) constitutes a large part of the ration. However, blood Cu levels did not reveal deficiencies. On the other hand, blood Cu levels are not very reliable indicators of Cu deficiency [90] (see 10.6). Liver Cu levels in slaughter cows from the same area were relatively low (on average 166 ppm (DM); ± 12% of the values <25 ppm (DM); mean values for the whole country ranging from 65-412 ppm (DM)) [17].

4.1.1.1 Quantitative significance of the hepatic Cu stores.

Copper requirements are determined by the endogenous losses and the Cu needs for milk production and growth. Besides this, Cu storage in the liver is important to cope with daily fluctuations in Cu supply. The amount of Cu stored in the liver can be calculated from the hepatic DM-weight and Cu concentration. Copper distribution through the liver tissue is more or less homogeneous when hepatic Cu concentrations are high (>1200 ppm (DM) [1]),

whereas distribution can be homogeneous [78] or not [16] if hepatic Cu concentrations are low (25-30 ppm (DM)). Regarding these observations, assessment of the total amount of liver Cu from relatively small liver samples will be most reliable in the case of high liver Cu concentrations.
The DM-weight of the liver can be assessed using the equation:

DM weight of the liver (g) = *a* x BW (kg) [11]. (IV)

This equation is based on carcass analysis data. The value of *a* lies between 5.3 and 3.8 and is inversely related to BW and growth. However, as it is not clear which of these values has to be used for mature dairy cattle, the average of these two values (i.e. 4.6) seems reasonable. Another experiment with cattle (BW 98-419 kg) resulted in a slightly different equation for the calculation of the DM weight of the liver [88].

DM weight of the liver (g) = 300 + 3.3 x BW (kg). (V)

Regarding the body weights this equation has possibly been derived from data of growing cattle. Nevertheless, no correction is made for age or growth rate. Using equation IV, the DM-weight of the liver of a 650-kg cow is 2.5-3.4 kg, i.e. on average ± 3.0 kg. Using equation V, the DM-weight of the liver of such a cow is 2.4 kg. A value of 2.7 kg for the DM-weight of a 650-kg cow seems, therefore, defendable.

For sheep, the DM weight of the liver can be calculated using the equation (18 lambs, r^2=0.81) [51]:

DM weight of the liver (g) = 90.79 + 0.9439 x BW (kg). (VI)

As to what extent equation VI is also valid for goats, is unclear.
As an example, the significance of liver Cu stores in cattle is calculated. If the hepatic Cu concentration is very low (20 ppm (DM)) [106] (cattle with liver Cu concentrations <30 ppm (DM) not being able to maintain normal plasma Cu concentrations [90]) the liver will contain ± 55 mg Cu. In a Dutch survey (14 cows) bovine livers appeared to contain on average 47.5 mg Cu/kg wet weight. Assuming the DM content of bovine livers is 25 [98] to 29% [14], the total amount of hepatic Cu in the Dutch survey was ± 475 mg/cow. On the other hand, hepatic Cu concentrations of ± 400 ppm (DM) are considered to be the upper limit of adequate concentrations ([106], no background data given). Then, the total amount of Cu will be ± 1080 mg.
Using these data, the relevance of the Cu stores can be assessed. An adult 650-kg cow producing 40 kg of milk needs 4.6 + 1.6 = 6.2 mg absorbed Cu (endogenous loss + milk). Assumed that (1) the ration contains no copper, (2) the liver of this cow contains 400 ppm Cu (DM), (3) this would decrease to 20 ppm (DM), (4) all hepatic Cu is available for the cow and (5) the endogenous losses do not change depending on the amount of Cu present in the liver, then the liver Cu store is sufficient to cover the needs of the animal during maximally ± 23 weeks on a severely Cu-deficient ration. If the initial concentration is 100 ppm (DM), then the hepatic amount of Cu is only sufficient for maximally 3.5 weeks. For a cow yielding 20 kg of milk, these periods are 22 and 4.5 weeks, respectively.

4.1.1.2 Depletion rate of Cu from the liver.

In calves (18-21 animals, 74-125 kg BW) on a semi-synthetic diet (1.0-1.4 ppm Cu (DM)), the depletion rate of Cu from the liver was described by the following equation [88;117]:

$Cu_{t1} = Cu_{t0} \times e^{-k(t1 - t0)}$ (VII)

in which Cu_{t0} = liver Cu concentration on day t0 (ppm (DM))
Cu_{t1} = liver Cu after (t1 – t0) days (ppm (DM))
k = 0.0273 ± 0.0008

Thus, the rate of decline of the liver Cu concentrations depends on the amount of Cu present in the liver. As this rate exceeded the Cu requirement for growth, the authors concluded that the endogenous Cu losses are also related to the amount of hepatic Cu. This dependence of the rate of decline of liver Cu concentrations from initial Cu concentrations is in accordance with other results [128]. However, as hepatic Cu concentrations in calves of 117 kg BW and growing 0.76 kg/d fell from 100 to 48 ppm (DM) in not more than 20 days, equation VII as well as the considerations in paragraph 4.1.1.1 on the quantitative relevance of hepatic Cu stores may not be valid under all conditions [128]. When assessing the endogenous losses, the ARC [11] does not take into account the initial amount of Cu present in the liver. The fixed value for endogenous Cu loss as given by the ARC (7.1 µg/kg BW) has, therefore, to be used with caution. The same remark may, however, be valid for the value of 4 µg/kg BW.

In beef heifers consuming prairie hay (3 g S/kg, 2.3 ppm Mo and 1.5 ppm Cu), after 60 days the liver Cu content had decreased from ± 55 to 38.9 ppm (DM)[12]. After 45 days repletion with Cu (either as sulphate or proteinate) the liver Cu content had increased to 88.9 or 106.6 ppm (DM) (difference not significant). While the Cu concentration in the ration was comparable to that used in the calves, both depletion and repletion occurred at a much slower rate than in the calves. Therefore, it is not clear as to what extent equation VII can also be used for beef or dairy cattle on usual roughage / concentrate rations.

In milk-fed animals, hepatic Cu retention may be equivalent to about 50% of the dietary Cu intake [11]. In ruminating lambs (9 months of age, 38 kg BW, 125 days on experiment), hepatic Cu retention declined from 2.0 to 0.3% (Ile de France) or from 1.3 to 0.2% (Merino) of dietary Cu intake when dietary Mo additions were raised from 0 to 8 ppm. The non-supplemented ration contained 30 ppm added Cu and 5 g added S/kg (mean daily intakes 36.3 mg Cu and 3.7 g S/lamb) [51]. In housed Scottish Blackface lambs, liver Cu concentrations were related to the daily Cu intake as described by the equation [53]:

Liver Cu (ppm (DM)) = 109.3 + 23.54 x mean daily Cu intake (mg) (VIII)

($p = 0.0001$; 43 groups of lambs; 16-20 weeks experimental period).

4.1.2 Beef cattle.

According to the NRC [97] the dietary Cu requirement of beef cattle is 10 ppm, but can range from 4 to over 15 ppm (depending on dietary Mo and S concentrations). This amount should be sufficient when dietary S and Mo concentrations do not exceed 2.5 g/kg and 2 ppm, respectively. For cattle grazing pastures containing 3-20 ppm Mo, dietary Cu concentrations of 7-14 ppm were inadequate. In feedlot diets containing a relatively large proportion of concentrates, Cu may be more available and, hence, the Cu requirement may be less than 10 ppm.
Moreover, breed differences may exist, Simmental and Charolais cows being more prone to Cu deficiency than Angus cows. However, as yet there is no reason to carry out separate calculations for Cu requirements of beef cattle of different breeds.
Recently, an equation for the assessment of dietary Cu requirements of beef cattle related to dietary Mo and S concentrations was derived from meta-analysis of literature data by Jongbloed et al. (Mo = ppm (DM) and S = g/kg DM) [69]:

Cu (ppm (DM)) = (0.309 + Mo x 0.042 + S x 0.311)/(0.095 – 0.005 x Mo) (IX)

At any dietary Mo and S concentrations, this equation calculates the dietary Cu concentration at which the liver Cu concentration remains constant for a period of 100 days.
Unfortunately, it was not possible to derive equations for dairy cattle (too few data available) and sheep (no statistical significant outcome, maybe due to breed differences). Although the use of this equation is attractive, this precludes a factorial approach of Cu requirements. Therefore, the factorial approach is also used to calculate beef cattle requirements.

4.2 Sheep

Calculation of the Cu requirements of sheep is essentially similar to that of cattle (same values/calculations for A_{Cu} (equation IIIa) and endogenous Cu loss). However, for breeds sensitive to Cu deficiency, the calculated values may be too low, for breeds sensitive to Cu toxicosis (e.g. Texel breed) these values may be too high. Endogenous loss (metabolic faecal + urinary losses) is estimated to be 4 µg/kg BW/day, although it may vary with the Cu status of the animal. Copper accretion in growing body tissues is assessed to be 0.5 [140][15] or 1.1 [45b][16] mg/kg gain[17]. A mentioned, a value of 0.5 mg/kg gain is adopted for all ruminants.
For pregnant animals, net Cu accumulation of uterine contents was estimated to be 15, 85 and 186 µg/day during the first, second and third trimester [93]. It is not clear as to what extent these estimated Cu accretion values are valid for twin foetuses. However, these values allowed for the build-up of foetal Cu levels (44 ppm (or ± 160 ppm (DM))) within marginal bands for newborn calves (50-200 ppm (DM)[140]) and can, therefore, not be truly classified as “minimal”.
Lactating ewes secrete 0.24-1.20 mg Cu/kg of milk in early lactation, whereas 0.11-0.32 mg Cu/kg of milk is secreted in late lactation [11; 151]. In another report, a value of 0.49 ppm is reported[18] [152]. Arbitrarily, to avoid extremely high Cu requirements for sheep the lowest value (0.11 mg/kg) is adopted. Mature milk of Massese sheep contains 0.52 mg/kg. This concentration does not differ significantly between parities [23].
Finally, wool contains on average 5 mg/kg DM. Therefore, in low-yielding breeds as the Welsh Mountain wool production will require a net amount of 5 mg Cu/year, whereas high-yielding breeds as the Merino will need a net amount of 18 mg [11]. On average, daily Cu need for wool growth is assessed to be 0.03 mg/day.

4.3 Goats

Factorial estimation of Cu requirements of goats is hampered by a lack of data. As no separate information is available on A_{Cu}, endogenous Cu loss or Cu content of growing tissues or pregnancy, for the parameters mentioned the values for cows and sheep are used. Thus, for calculation of A_{Cu} equation IIIa is used, and for endogenous Cu loss and Cu content of growth values of 4.0 µg/kg BW and 0.5 mg/kg growth are adopted, respectively. For pregnant goats, the values for sheep will be used (i.e. 15, 85, 186 µg/day). For the last trimester of pregnancy, a rounded value of 0.19 mg/day is adopted.
Goat colostrum contains (mg Cu/kg) 1.2 (day 1) to 0.6 (day 2), whereas mature goat milk contains (mg Cu/kg) 0.07 [14; 28], 0.12 [82], 0.173 [108], 0.22 [15] or 0.39 [101]. The 0.39 mg/kg value is considered to be an abberating high value, which is excluded from the calculation. The average of the remaining values for mature milk is 0.15 mg Cu/kg. As no

15 Origin of value unclear.
16 Derived from a comparative slaugther experiment.
17 Breeds not given.
18 Maize silage/barley diet containing (DM) 4.5-4.9 ppm Cu, 0.9 ppm Mo and 1.4-1.5 g S/kg.

data are available on Cu content of goat hair, for fibre-producing goats the value for sheep (0.03 mg Cu/day) is adopted.
No data are available on differences in Cu requirements between breeds, and especially for fibre-producing goats (Cashmere and Angora) information is lacking [6].

4.4 Conclusion

The following equation can be used to calculate the required Cu-concentration of ruminant rations:

$$C = \frac{(BW \times a) + (kg\ milk \times b) + (kg\ growth \times 0.5) + c}{A_{Cu} / 100 \times DMI}$$

in which C = required dietary Cu concentration (ppm (DM))
BW = body weight (kg)
A_{Cu} = true Cu absorption (%), calculated using the above equation IIIa and assuming these equation can be used to calculate A_{Cu} of the total ration (see 10.3.2.1.4)
DMI = dry matter intake (kg/day).
endogenous Cu loss = 0.0071 mg/kg BW/day
a = endogenous Cu loss = 0.0071 mg/kg BW/day (dairy cattle and beef cattle) or 0.004 mg/kg BW/day (sheep and goats)
b = Cu concentration of milk (mg/kg)
c = amount of Cu needed for pregnancy (mg/day)

For dairy cattle, b = 0.04 mg/kg, c increases from 0.61 to 2.07 mg/day (from 140 to 281 days of pregnancy, respectively) [11]. For the last weeks of pregnancy, a rounded value of 2 mg/day is adopted.
For sheep, b = 0.11 mg/kg, c = 0.015, 0.085 or 0.186 mg/day for the 1st, 2nd or 3rd trimester of pregnancy, respectively [11]. For the last trimester of pregnancy, a rounded value of 0.19 is adopted.
For goats, b = 0.15 mg/kg, for c see values assumed for sheep.

5 ALLOWANCES

The allowance for a certain animal category (e.g. adult, lactating sheep) is the gross requirement plus a safety margin. In this report, the safety margin is arbitrarily set at 50%.

5.1 Cattle

Using the above equations and assumptions, some examples of minimal dietary and allowances (including a safety margin of 50%) have been calculated (Table 8).

Table 8 Examples of calculated Cu requirements and allowances

Category	DMI (kg)	Requirement (mg/day)	Safety factor	Allowance mg/day	Allowance ppm (DM)
Growing female cattle					
4 months, 850 g growth/day, 130 kg BW	3.9	37	1.5	56	14.4
9 months, 700 g growth/day, 250 kg BW	5.6	61		92	16.4
16 months, 625 g growth/day, 400 kg BW	7.3	88		132	18.1
Dairy cattle (650 kg BW)					
Cow, dry, pregnant, 8-3 wk a.p.	11.5	184		277	24.1
Cow, dry, pregnant, 3-0 wk a.p.	11.0	184		277	25.2
Cow, lactating, 20 kg of milk	18.5	150		227	12.2
Cow, lactating, 40 kg of milk	23.5	173		260	11.1
Beef cattle, intermediate type					
1000 g growth/day, 100 kg BW	3	34		51	16.9
1200 g growth/day, 250 kg BW	6	66		99	16.6
1100 g growth/day, 500 kg BW	9	114		172	19.1
Veal calves					
1150 g growth/day, 150 kg BW	4.5	46		69	15.2
1400 g growth/day, 275 kg BW	7	74		111	15.9
Sheep (75 kg BW)					
High sensitivity to Cu poisoning					
Growing lamb, 0.3 kg growth/day, 40 kg BW	1.6	9	1	9	5.4
Sheep, pregnant, last trimester	1.9	14		14	7.2
Sheep, lactating, 3 kg of milk, nursing 2 lambs	2.6	18		18	6.8
Intermediate sensitivity to Cu poisoning					
Growing lamb, 0.3 kg growth/day, 40 kg BW		9	1.5	13	8.1
Sheep, pregnant, last trimester		14		20	10.7
Sheep, lactating, 3 kg of milk, nursing 2 lambs		18		26	10.1
Low sensitiviy to Cu poisoning					
Growing lamb, 0.3 kg growth/day, 40 kg BW		9	2	17	10.8
Sheep, pregnant, last trimester		14		27	14.3
Sheep, lactating, 3 kg of milk, nursing 2 lambs		18		35	13.5
Goats (70 kg BW)					
goat, pregnant, last trimester	1.7	13	1.5	20	11.6
goat, lactating, 4 kg of milk	3.2	24		37	11.5

For non-ruminating calves, A_{Cu} varies with age and is estimated to be on average 70% [11]. Using this value, minimal Cu requirements will rarely exceed 1 ppm (DM). On the other hand, allowances of 1.2 [11] to 2 ppm Cu (DM) are recommended [61]. No remark is made as to “minimal” or “optimal”. However, these values seem to be defendable.
For veal calves consuming a diet consisting of a milk substitute and up to 1.5 kg maize silage or 0.5 kg grains (6 months of age), no data are available to reliably assess their Cu allowance.

For dry, pregnant cattle during the last stage of gestation, no qualification of the Cu accretion in the pregnant uterus can be given (see paragraph 4.1). Therefore, qualification of this allowance is precluded.
On the other hand, requirements of 7.0-30.0 ppm Cu (DM) (A_{Cu} = 6-1.5%; BW = 500 kg) have been calculated for cows during the last stage of gestation [140]. These calculated values are similar to those presented in Table 8 (BW = 650 kg). However, as these high estimations are reported to allow for build up of a large foetal reserve of Cu, these values cannot be regarded as “minimal”.

5.2 Sheep

The preruminant lamb also absorbs Cu very efficiently: A_{Cu} is estimated to be 90% [11]. Minimal dietary Cu requirement will, therefore, hardly attain 1 ppm (DM). Thus, the suggested allowance of 1 ppm Cu (DM) [61] can be adopted.

For pregnant and lactating ewes, the factorial estimations of Cu requirements as given by Underwood and Suttle [140] can be used. As argued in 4.2, Cu accretion values used for the pregnant uterus are not truly minimal. However, using similar values, Underwood and Suttle [140] regard their calculated requirements to allow for the build up of a large foetal reserve of Cu. As this statement is not clarified, judgement is precluded.
For A_{Cu} values of 1.5-6%, pregnant ewes (last trimester of gestation, carrying twin foetuses), Cu requirements would be 21.0-7.0 ppm (DM). For lactating ewes, Cu requirements would be 23.2-5.8 (1 kg of milk) to 28.4-7.1 ppm (DM) (3 kg of milk). However, both during the last stage of gestation and during lactation the supply of concentrates is necessary in most cases, thereby increasing A_{Cu}. Therefore, although a rounded allowance of 31 ppm Cu (DM) (including a safety margin of 10%) for lactating ewes is proposed, in most cases requirements will be substantially lower. In a summary, it is impossible to give one dietary Cu allowance for all sheep breeds.

5.3 Goats

For goats, a deficiency limit of 7 ppm Cu (DM) is suggested, whereas the requirement is suggested to be 8-10 [6; 15; 73] or even 10-20 ppm (DM) [6]. However, in the same reference the latter upper limit is already suggested to be the maximum tolerable level for goats [6]. No qualifications (“minimal” etc.) of these data are given.

6 CRITERIA TO JUDGE COPPER STATUS

6.1 Introduction

To carefully discern the positive and negative effects of supplying a certain amount of Cu, criteria are needed to judge if requirements are met or certain beneficial effects exerted. Moreover, these criteria can reveal causes of poor performance.

6.2 Characterization of suitable indicators

To carefully judge the Cu status of animals, suitable indicators are required. Such indicators react sufficiently rapid and yield information on variations in dietary Cu supply. An indicator can reflect the actual supply (during the past days to weeks) or the historical supply (during the last months to years). Indicators supply data as to what extent the available amount of Cu is limiting or excessive for optimal performance and health. This approach can substantially differ from the clinical one. From a clinical point of view, dietary Cu supply may be sufficient when the animals do not show any clinical deficiency symptoms. However, it is very well possible that such an animal performs better (e.g. higher growth rate) in case of an extra dietary Cu supply.
Suitable indicators should be sufficiently sensitive to variations in dietary trace mineral supply (preferably to both excess and insufficient supply), be sufficiently specific (reacting only on variations in one mineral), and be readily accessible.

6.3 Dietary Cu, Mo, S and Fe concentrations

To assess the Cu supply from the ration, at least Cu and Mo concentrations should be determined, supposing the S content is sufficiently high to evoke a Cu x Mo x S interaction. The NRC [96] recommends a rough schedule for the Cu content of the ration of sheep, as presented in Table 9.

Table 9. Recommended dietary Cu concentrations at different dietary Mo concentrations for sheep, according to the NRC (1985)

Mo	**Cu**		
Ppm	**ppm (DM)**		
	Growth	Pregnancy	Lactation
< 1.0	8-10	9-11	7-8
> 3.0	17-21	19-23	14-17

However, as outlined by Underwood and Suttle [140], there is little agreement as to “critical” Cu:Mo ratios. Ratios of 2:1 (cattle) to 4:1 (sheep) have been suggested to be minimal to avoid Cu deficiency symptoms. Marginal bands are given in Table 10.

Table 10. Marginal bands [a,b] for dietary Cu:Mo ratios (according to Underwood and Suttle (1999))

Forage type	**Cattle and sheep**	**Goats**
Fresh grass	1.0-3.0	0.5-2.0
Roughage [c]	0.5-2.0	0.3-1.2

[a] values below marginal band: high risk of deficiency; above marginal band: beneficial effect of Cu supplementation is not likely; [b] limitations: dietary S >2 g/kg DM and dietary Mo < 8 ppm (DM); [c] not specified; no data on grass and maize silage

Using solely the Cu:Mo ratio does not account for: (1) the influence of dietary S (and Fe) on Cu absorption and (2) possible differences between the actual and the previous Cu supply and (3) differences in the Cu x Mo x S interaction in different roughages. On the other hand, the correct determination of S is the most difficult and expensive among these elements, whereas in green swards (on which Cu deficiency mainly occurs), S is usually present in sufficient quantities to allow for the occurrence of the Cu x Mo x S interaction. In some areas, the contribution of S from drinking water has also to be accounted for, but drinking water analyses are mostly not available [99]. Finally, the Cu x Mo x S and Cu x Fe interactions are not simply additive (see 3.2.2). Marginal bands for Cu:Fe ratios of 50-100 have been suggested [140], but Fe effects on Cu absorption appear to occur only at low dietary Mo concentrations (see 3.2.2). Thus, if dietary Mo concentrations are relatively high (5-6 ppm (DM)), the dietary Cu:Mo ratios (Table 10) can be used to roughly assess the risk of Cu deficiency or toxicity. If Mo concentrations are low (0.1 ppm Mo (DM)), Cu:Fe ratios can be used.

6.4 Ranking criteria for indicators of Cu status

When comparing different Cu sources, bioavailability has to be related to a reference mineral source. This source has per definition a relative bioavailability of 100%. For Cu, the reference source is $CuSO_4.5\ H_2O$ (reagent grade). Several criteria are used to judge the effect of supplying a certain amount of Cu. However, not all criteria are equally important. Therefore, criteria have to be ranked in order of their importance. This order may be different for the specific animal species or even category. Beside this, it is important to note that the order of importance may depend on the level of supply (below or above recommended requirements). Further, if more criteria are available, weighing factors have to be used to obtain a final score [68]. Criteria and weighing factors are presented in Table 11.

Table 11 Ranking of criteria to judge the effects of a certain Cu supply on cattle performance as proposed by Jongbloed [68]

	Ranking of importance (weighing factors)	
Criterion	Cu supply below requirements	Cu supply above requirements
Cu absorption	2	1
Hepatic Cu content	4	3
Superoxide dismutase activity	4	1

As an example, the relative bioavailability of two Cu sources[19] can be calculated ($CuSO_4.5\ H_2O$ set at 100%)

	hepatic Cu content	Cu absorption
source A	80	70
source B	75	60

The relative bioavailability of source A = 4 x 80 + 3 x 70 = 530/(4+3) = 76%.
The relative bioavailability of source B = 4 x 75 + 3 x 60 = 480/(4+3) = 69%.

Besides these indicators, plasma Cu has some limited value for the assessment of low Cu status. When liver Cu reserves are severely depleted (<20-50 ppm (DM)), plasma Cu concentrations are often very low (< 6-7 µM). [26]; [123]; [148] (Table 4). However, the use of plasma Cu for the determination of Cu status has several disadvantages:

- even at severely deficient rations (1.1 ppm Cu (DM) plasma Cu concentrations only slowly decline through 3-4 months to values < 6 µM [39]

[19] Cu supply above requirements.

- even at liver Cu levels < 20-30 ppm (DM) blood Cu levels up to 22 µM can occur, whereas no clinical abnormalities need to be observed [148] (Table 7)
- variable interval between onset of low plasma Cu concentrations and clinical symptoms [90]
- differences in plasma Cu concentrations due to breed and age can occur within one (dairy) herd at the same dietary Cu concentration [33]
- the plasma Cu concentration can be influenced by infections and fluctuations of oestrogen concentrations in the blood [92].

6.5 Possible indicators of Cu status

As the liver is the main storage organ for Cu in the body, determination of liver Cu concentrations has been proposed to be the main Cu status indicator [140]. For Cu, Delves [30] discusses the use for humans of plasma Cu, plasma Cu proteins and Cu-containing enzymes, erythrocyte Cu, and Cu-loading tests. According to this author, elevated plasma Cu concentrations are of little value, as many clinically abnormal conditions are associated with hypercupremia. Low plasma Cu can point into the direction of an insufficient dietary Cu supply, but can also be associated with inherited diseases of Cu metabolism. However, at least in cattle the course of plasma Cu is not closely related to variations in dietary Cu supply [90]. Moreover, in contrast to man, both in cattle and small ruminants liver Cu samples can easily be obtained [138; 139]. Excess absorbed Cu is stored in the liver, and can be mobilized in case of a suboptimal Cu supply [67;68]. In animals, caeruloplasmin is considered as an acute phase protein. Due to several causes (e.g. infection) its concentration can vary considerably, and therefore also this protein is not sufficiently valuable to assess Cu status. Erythrocyte Cu concentration and plasma enzyme activities such as superoxide dismutase do not react sufficiently rapid to variations in dietary Cu supply. The determination of superoxide dismutase activity is variable between different assay methods. Moreover, erythrocytes have to be washed (removal of plasma inhibitors) and diluted before assaying, which may hamper a proper interpretation of the results. Therefore, contrary to the ranking as proposed in Table 11, superoxide dismutase seems not to be a suitable indicator of Cu status. As superoxide dismutase activity and erythrocyte Cu concentrations are well correlated [140], erythrocyte Cu may be just as informative and less problematical. However, as no data are available on correlations between liver Cu and erythrocyte Cu concentrations, it is not clear as to what extent the determination of erythrocyte Cu can replace that of liver Cu concentrations. Finally, cytochrome oxidase of leukocytes is too labile, causing analytical problems [90].

6.6 Conclusions

To judge the Cu status of cattle, determination of liver Cu concentrations is the parameter of first choice [138]; when Cu deficiency is suspected plasma Cu can be used for screening purposes (values < 6-7 µM are indicative for deficiency). However, the limitations of this parameter should be taken into account. For bovine livers, Cu levels of 100-400 ppm (DM) (25 – 100 ppm wet weight) are considered normal [106]. Liver Cu concentrations <20-50 ppm (DM) are considered to indicate for deficiency [17; 27; 33; 90; 98]. A review of these response parameters has been given by Delves [30]. Mainly the non-supplemented animals (yearlings and heifers) should be sampled. As Cu absorption from fresh grass is lowest, the poorest Cu status will occur by the end of the grazing period. As a sufficient Cu status during the grazing period will not automatically implicate a sufficient status by the end of this period, spring and summer liver Cu data have to be judged differently from fall data [27].

7 DEFICIENCY

Due to the relatively low Cu concentrations in forages (Table 2) and the vulnerability of Cu absorption to impairment by S and thiomolybdates, Cu deficiency can occur in ruminants. Early signs are hair depigmentation around the eyes as well as bleaching of black and red coat, and diarrhoea. Moreover, anaemia, fragile bones, osteoporosis, osteochondrosis, widening of epiphyses, cardiac failure, poor growth, reduced fertility and immune function can be observed in all kinds of Cu-deficient ruminants.

In sheep, an early sign of Cu deficiency is the loss of the wool “crimp”, resulting in straight, “hair-like” wool. Neonatal ataxia (“swayback”) in newborn lambs and goat kids is another clinical sign of Cu deficiency, which cannot be cured but only prevented by Cu supplementation [97; 98; 140; 155].

In sheep, considerable breed differences exist. Selected data on differences in hepatic Cu accumulation between breeds are given in Table 12.

Table 12. Selected data on breed differences in hepatic Cu accumulation of sheep [a]

Ref.	Breed / cross	N	Dietary Cu (ppm)	Weeks	Liver Cu (ppm (DM))	% retention[b]	Result
[153][c]	SB x SB	78	12 (DM)	13	214	5.6	T and S > SB EF and FL intermediate
			20		384		
	EF x SB		12		278	6.7	
			20		437		
	FL x SB		12		284	8.6	
			20		418		
	S x SB		12		397	7.3	
			20		635		
	T x SB		12		352	13.7 D	
			20		676		
[85] [d]	Sk	17	48	9	805-2020		T > Sk
		4	69		1065-1260		
		3	68		1305-1695		
	T	3	48		980-2625		
		3	69		1600-1935		
		20	68		1290-2735		
[154]	SB	82	4 (DM)	28	16		WM > SB
	SB x WM				51		
	WM				34		
	SB		9		92		
	SB x WM				243		
	WM				226		
	SB		17		231		
	SB x WM				325		
	WM				415		
	SB		29		271		
	SB x WM				444		
	WM				486		
[111]	T	7	22 [d,e]	14-15	1652		T > FM; cross intermediate
	T x FM	6	22		1238		
	FM	7-8	25		1076		
[149]	SB	193	? (pasture)	24	25		WM > C, SB
	C				32		
	WM				66		
	SB x C				35		
	SB x W				38		
	C x W				42		
[51]	SAMM	33	30 [d]	18	81	1.3	IF > SAMM
	IF	30			111	2.0	

[a] C = Cheviot; EF = East Friesland; FL = Finnish Landrace; FM = Friesian Milksheep; IF = Ile de France; S = Suffolk; SAMM = South African Mutton Merino; SB = Scottish Blackface; Sk

= Schwarzkopf; T = Texel; WM = Welsh Mountain; [b] estimated proportion of ingested Cu retained by the liver after 13 weeks; [c] rams of the different breeds mentioned mated to SB ewes; [d] estimated dietary Cu concentrations; [e] 2 animals died from chronic Cu toxicosis; D = significantly higher than SB x SB

Moreover, Cu-deficient North Ronaldsay sheep showed rapidly increasing plasma Cu concentrations after oral Cu repletion, whereas Scottish Blackface sheep showed no and Welsh Mountain showed moderate increases of plasma Cu concentrations at similar oral Cu repletions [150]. However, as the plasma Cu concentration is not a suitable indicator of Cu status, these observations have to be interpreted with caution. Although the inventory shown in Table 12 is far from complete, the extreme tendency of the Texel breed to accumulate Cu is clear. On the other hand, the Scottish Blackface breed in all cases has the lowest concentrations. The latter breed may, therefore, be one of the most susceptible ones to develop a Cu deficiency.
Data on differences in Cu metabolism in cattle are scarce. Selected experimental results are given in Table 13.

Table 13. Selected data on differences in Cu metabolism in cattle breeds

Ref.	Breed	Category	n	Dietary Cu (ppm)	Weeks	Liver Cu (ppm DM))	Result
[33]	Holstein	Lactating / growing	2	5	8.5	167	Holstein = Jersey
	Jersey		2			172	
	Holstein		2	80 [a]		439	
	Jersey		2			520	
[120]	Holstein	Lactating	6	8	8	222	
	Jersey		6			272	
	Holstein		6	35 [b]		562	
	Jersey		6			656	
[34]	Holstein	Steers	10	66	43-49	368	
	Jersey		10			490	

[a] extra Cu from $CuSO_4$ or Cu proteinate (mean value); extra Cu from $CuSO_4$;

According to the results presented in Table 13, no significant differences in Cu metabolism exist between Holstein and Jersey cattle. On high-Cu rations, however, Jersey cattle tend to accumulate some more Cu in their livers. In beef cattle, differences in Cu metabolism between Angus, Simmental and Charolais cattle have been investigated. Simmental heifers fed a diet containing either 5 or 40 ppm Cu (DM) for 2 months appeared to excrete significantly more Cu via the bile than did Angus heifers [45]. Angus heifers and their calves tended to have higher plasma Cu concentrations than their Simmental and Charolais counterparts, whereas Angus steers had slightly higher plasma Cu concentrations, apparent Cu absorption and Cu retention than Simmental steers [143]. The rations contained 4-4.5 ppm Cu (DM) (heifers) or 9 ppm Cu (DM) (steers), respectively. The experiments lasted for 40 weeks (heifers) or 4-7 weeks (steers). In another experiment, no significant differences in liver or plasma Cu concentrations of heifers from either Angus, Hereford or Simmental sires and Hereford dams could be demonstrated [118]. The ration was assessed to contain ± 5 ppm Cu (DM) and was fed to the animals during 2-3 months. However, as both plasma Cu concentrations and biliary Cu excretion are unreliable indicators of Cu status [68], the beef cattle experiments do not supply convincing evidence as to breed differences in Cu metabolism.
In summary, most differences in Cu metabolism between cattle breeds are minor and of no practical significance.

7.1 Direct measures in deficiency cases

7.1.1 Direct continuous supplementation

For grazing animals, extra Cu can be provided by the use of salt licks containing 0.5-1.9% Cu (from e.g. $CuSO_4$) [140]. However, the individual variation in salt intake between animals may impair the reliability of this way of Cu supply.
Copper sulphate can be dissolved in water and sprinkled over the forage to supply amounts of 5-10 ppm (DM). It is also possible to add 2-5 mg Cu/L of drinking water using a proportioning device [140]. However, the latter method entails the risk of Cu toxicity in susceptible animals [27].

7.1.2 Direct discontinuous supplementation

As animals store Cu in their livers when intake exceeds requirements and mobilize this Cu when dietary supply is inadequate, discontinuous supplementation is often sufficient. Copper sulphate drenches at monthly or longer intervals may be satisfactory in many cases. In cases of high dietary Mo concentrations (>5 ppm (DM)) daily Cu supplementation may be necessary. However, under Dutch circumstances such high Mo concentrations are rare (Table 2). In flocks of sheep suffering from swayback, all lambs can be treated with an oral dose rate of 1 mg / kg BW. Subcutaneous or intramuscular injections of Cu glycine, CuCaEDTA and Dicuprene constitute another suitable way of Cu administration. Doses of 30-40 mg Cu for sheep and 120-240 mg Cu for cattle have been reported to be sufficient at 3-month intervals. Copper heptonate ($2C_7H_{13}O_8$ $Cu.2H_2O$) and Cu hydroxyquinoline sulphonate (cupric-bis-8-hydroxyquinoline 5-7 disulphonic acid salt of tetra diethylamine, 6.05% Cu) cause less tissue irritation than the former preparations, but pose a greater risk of acute toxicity. Methionate complexes are the least toxic, but cause severe tissue irritation [140].

7.1.3 Slow release oral supplementation

For longer periods, Cu can be supplied in the form of CuO needles. Needles containing CuO and CuO powder are equally effective. Copper is then released slowly during several weeks and liver Cu stores can be increased over several months. However, diarrhoea and abomasal parasitism can impair the efficacy of this way of Cu supplementation. Therefore, anthelmintic treatment several weeks before turnout is recommended as to maximize efficacy of Cu supply [140].

8 TOXICITY

8.1 General

Excess Cu can originate from pollution of the pasture, e.g. the application sewage sludge [52], whereas the current importance of Cu from pig slurry (an important source of Cu in the past) is minor due to the extensive reduction of (growth-promoting) dietary Cu levels applied in pig husbandry (A.W. Jongbloed, personal communication). Further, Cu toxicity in susceptible breeds can arise from consumption or application of any Cu-containing feed or supplement, such as salt licks or concentrates for cattle [140].
Copper toxicity may most easily develop in susceptible sheep breeds [140]. Copper toxicity symptoms are essentially the same in cattle, sheep and goats. First signs of Cu toxicity are rather unspecific (decreased feed intake, reduced weight gains, dullness, diarrhoea, dark urine, anaemia, jaundice). Ruminants suffering from chronic Cu toxicity often have liver Cu levels >1000 ppm (DM). Sheep, however, can show histological and biochemical evidence of liver damage at liver Cu levels of 350 ppm (DM) [140]. Following chronic accumulation of Cu in the liver, suddenly a haemolytic crisis can occur. This condition is characterized by haemolysis, methaemoglobinemia, haemoglobinuria and jaundice. Serum aspartate aminotransferase (ASAT), glutamate dehydrogenase (GDH) and lactate dehydrogenase (LDH) activities are increased 5-8 weeks before the haemolytic crisis occurs [5;140]. During the development of chronic Cu intoxication in calves, ASAT activities rose from ± 15 to ± 1800 IU/L, whereas LDH activities rose from ± 900 to 16000 IU/L (no GDH activities given) [146]. In most cases, animals die within a short time. Pathologic symptoms mainly include yellow to orange discoloration of liver and carcass and liver necrosis [11; 47; 59; 140; 146].
The ingestion of pyrrolizidine containing plants such as heliotrope (*Heliotropium europaeum*) can result in liver damage and excessive Cu accumulation in the liver of sheep and, consequently, to Cu toxicosis [31; 55]. However, heliotrope does not occur in the Netherlands. Other pyrrolizidine containing plants (e.g. ragwort (*Senecio jacobaea*), that abundantly occurs in the Netherlands) do not cause excessive hepatic Cu accumulation in sheep. Therefore, under Dutch circumstances this phenomenon is of no practical value.

8.1.1 <u>Cattle</u>

Milk-fed male calves fed either 10, 50, 200, 500 or 1000 ppm Cu for 42 days showed deterioration of weight gains and feed efficiency when fed 200 ppm Cu or more. Only 4 of 7 calves survived the 1000 ppm treatment, the succumbing ones showing typical signs of chronic Cu toxicity and haemolytic crisis [65]. Similar observations were made in 5 milk-fed calves fed 50-300 ppm Cu for 116 days. Only the 50-ppm calf survived, while the others died during a haemolytic crisis [146]. However, slight growth retardation has been observed in animals receiving only 5.5 ppm Cu (DM) [19].
Maximum allowed dietary Cu concentrations for ruminating animals largely depend on the levels of interacting components, mainly Mo and S. In young beef calves fed 115 ppm Cu for 91 days signs of Cu toxicity occurred [116]. Therefore, a maximum tolerable level of 100 ppm Cu for beef cattle is suggested [97]. For dairy cattle, this maximum tolerable level should be 40 ppm Cu, unless dietary Mo concentration is greatly elevated [98]. No more comments are made concerning this Mo level, nor the reason for the large difference between the maximum levels for beef and dairy cattle are discussed either.

8.1.2 <u>Sheep</u>

In sheep, considerable breed differences exist in sensitivity for Cu toxicosis (Table 12). Hepatic Cu retention in milk-fed lambs may be up to 50% of the dietary Cu intake. Growing lambs (breed not given), fed a ration containing only 8 ppm (DM), showed signs of Cu poisoning [11]. Moreover, hepatic Cu concentrations of Texel x Scottish Blackface lambs were as high ± 700 ppm (DM) after feeding a ration containing 12 ppm Cu (DM) for 13 weeks [153]. This hepatic Cu concentration is well within the toxic range [106] and entails the risk of animals dying from Cu toxicosis. Therefore, giving one maximum tolerable level for all breeds is impossible, although 15 ppm (DM) is suggested [6].

8.1.3 Goats

Pre-ruminant Angora goat kids appear to be very sensitive to Cu toxicosis [59]. A milk substitute ration containing approximately 10 ppm Cu (DM) caused 3 out of 24 kids to die from a haemolytic crisis. On the other hand, with respect to sensitivity to Cu toxicosis ruminating goats seem to be intermediate between sheep and cows. For ruminating goats, a maximum dietary concentration of 20 ppm Cu (DM) is recommended [6].

8.2 Direct measures in toxicity cases

The most convenient measure in toxicity cases in sheep is the application of three subcutaneous injections of 3.7 mg ammonium tetrathiomolybdate on alternate days [140]. Oral administration of 10 mg Mo (e.g. as 20 mg ammonium molybdate) + 5 g sulphate (from Na_2SO_4 or K_2SO_4) with the solid feed (to prevent rapid passage of the rumen and development of diarrhoea) per animal per day is also recommended. This treatment should be sustained for 2-4 months [52]. As this treatment causes an increase of TCA-insoluble Cu in plasma, determination of plasma Cu is not useful to control the effectiveness of the treatment. Therefore, superoxide dismutase determinations in plasma have to be carried out for this purpose. As the rest of the flock will be at risk either, all animals should be transferred to a diet low in Cu, such as a diet containing whole grains [140] and high in readily degradable protein [52]. When animals are fed hay or concentrates, turning the animals out to pasture can also be helpful [52]. Although penicillamine has been demonstrated to substantially increase urinary Cu excretion, the use of tetrathiomolybdate is less expensive and, moreover, directly influencing liver Cu stores by increasing biliary Cu excretion [43]. The addition of gypsum (15 g/kg DM) or sodium molybdate (19 ppm (DM)) to the forage may be helpful to prevent toxicity. Finally, if pasture is the source of Cu toxicity, fertilizing the pasture with sodium molybdate (0.27 kg/ha) can be used to lower the long-term Cu accumulation [140].

9 PREVENTION

9.1 Short-term prevention strategies

Both short- and long-term prevention strategies have been reviewed by Suttle [128; 140]. Short-term prevention of Cu deficiency is best performed using the oral route for Cu supply. A single oral dose of $CuSO_4$ is rather ineffective, as most of this bolus will be excreted. (Glass) boluses or CuO needles, which are retained in the reticulo-rumen, slowly release Cu and are a convenient and safe way of supplying Cu to ruminants. For animals of 300-500 kg BW, 50-100 gram can be applied.

9.2 Long-term prevention strategies

Supplementation of Cu via Cu-containing fertilizers raises the Cu content of the roughage. However, adequate levels are often much higher for the grazing animals (78 ppm Cu (DM)) than for the plants (< 4 ppm Cu (DM)). As the Cu given in excess is lost to the environment, this can cause unwanted accumulation of Cu in soils and ground water. Copper present in the soil only slowly disappears [52]. Moreover, the application of Cu-containing fertilizers on pastures for grazing dairy cattle can give rise to contamination of the udder and milk, leading to decreased oxidative stability of dairy products. Therefore, lactating dairy cattle should not graze such pastures within a period of two weeks after application of the fertilizer. Susceptible sheep breeds should be withheld from such pastures for 6 months (risk of intoxication) [27]. Soils with Cu-HNO_3 values >15-20 are hazardous with respect to Cu toxicosis in susceptible sheep breeds [52]. On the other hand, Cu fertilization is only useful when the soil Cu-HNO_3 value is <5. When this value is >5, the Cu content of the sward cannot be raised by Cu fertilization [27]. The effect of Cu fertilization is also soil-dependent. A single dressing of 3.5-6 kg Cu (e.g. as $CuSO_4$) per ha should be sufficient for 3-4 years [26a], but longer residual effects (>23 years) have been reported on sandy soils. Fertilizer treatment is not recommended on soils high in organic matter, as Cu becomes fixed and unavailable in humic acid complexes [140]. In the Netherlands, this is an important feature of peat soils. To adequately apply Cu-containing fertilizers, soil analysis each 4 years is recommended.

Table 14 Inventory of Cu allowances for cattle, sheep and goats as used in some foreign countries (ppm (DM))

		Allowance				
Country	**Ref.**	**Cattle**	**Ref.**	**Sheep**	**Ref.**	**Goat**
Great Britain	[68]	1-15	[140][c]	4.3-17.2 (lamb) 7.0-21.0 (gestation) 5.8-28.4 (lactation)	[6]	10-20
USA[a,b]	[98]	12 (300-kg heifer) 15.2 (500-kg heifer) 15.7 (650-kg cow, 40 kg of milk) 13.7 (650-kg cow, end of gestation) 10 (4-15) (beef cattle)	[96]	8-21[c] (growth) 9-23[c] (gestation) 7-17[c] (lactation)	[95]	?
Germany	[42]	10 (growing and mature cattle)		?	[13]	10-15 (adult)
France	[49]	10 (7 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
[c] depending on Mo and S concentrations of the ration

LITERATURE

[1] Abdelrahim AI, Wensing T, Schotman AJH. Distribution of iron and copper in the liver and spleen of veal calves in relation to the concentration of iron in the diet. Research in Veterinary Science 1986; 40: 209-211.

[2] Abdelrahman MM, Kincaid RL. Deposition of copper, manganese, zinc, and selenium in bovine fetal tissue. Journal of Dairy Science 1993; 76: 3588-3598.

[3] Ademosun AA, Munyabuntu CM. Sheep: metabolic interactions of copper with iron, molybdenum and sulphur. International Goat and Sheep Research 1982; 2: 13-21.

[4] Adrian J. Les éléments minéraux. In: Adrian J (ed.), Valeur alimentaire du lait. Paris: La maison rustique; 1973.

[5] Adrichem PWMv. Wijzigingen in de activiteit van serumenzymen en in het LDH iso-enzympatroon bij chronische koperintoxicatie van schapen. Tijdschrift voor Diergeneeskunde 90: 1371-1381.

[6] AFRC. AFRC Technical committee on responses to nutrients. Report No. 10. The nutrition of goats. Nutrition Abstracts and Reviews, B 1997; 67: 765-830.

[7] Ammerman CB, Henry PR, Miles RD, Garnsworthy PC, Wiseman J. Supplemental organically-bound mineral compounds in livestock nutrition. Recent advances in animal nutrition 1998 1998; 67-91: -91.

[8] Anke M, Hennig A, Groppel B, Lüdke H. Der Einfluß des Kadmiums auf das Wachstum, die Fortpflanzungsleistung und den Eisen-, Zink- und Kupferstoffwechsel Effect of cadmium on growth, reproductive function and the metabolism of iron, zinc and copper. Archiv für experimentelle Veterinärmedizin 1971; 25: 799-803.

[9] Anke M, Hennig A, Schneider HJ, Lüdke H, Gagern Wv, Schlegel H. The interrelations between cadmium, zinc, copper and iron in metabolism of hens, ruminants and man. TEMA 1970; 1: 317-320.

[10] Anke M, Masaoka T, Hennig A, Arnhold W. Antagonistic effects of a high sulphur, molybdenum and cadmium content of diets on copper metabolism and deficiency symptoms in cattle and pigs. TEMA 1987; 6: 317-318.

[11] ARC. Trace elements. In: Agricultural Research Council (ed.), Nutrient requirements of ruminant livestock. Londen: 1980: 221-262.

[12] Arthington JD, Corah LR, Blecha F, Hill DA. Effect of copper depletion and repletion on lymphocyte blastogenesis and neutrophil bactericidal function in beef heifers. Journal of Animal Science 1995; 73: 2079-2085.

[13] Ausschuss für Bedarfsnormen der Gesellschaft für Ernährungsphysiologie. Recommendations for the supply of energy and nutrients to goats. Frankfurt am Main: DLG Verlag; 2003.

[14] Benemariya H, Robberecht H, Deelstra H. Zinc, copper, and selenium in milk and organs of cow and goat from Burundi, Africa. Science of the Total Environment 1993; 128: 83-98.

[15] Berg GJvd, Yu S, Van der HA, Lemmens AG, Beynen AC. Dietary fructose vs glucose lowers copper solubility in the digesta in the small intestine of rats. Biological Trace Element Research 1993; 38: 107-115.

[16] Bingley JB, Dufty JH. Distribution of copper in the tissues of the bovine neonate and dam. Research in Veterinary Science 1972; 13: 8-14.

[17] Binnerts WT. De koperstatus van het rundvee in Nederland
Copper status of cattle in the Netherlands. Tijdschrift voor Diergeneeskunde 1986; 111: 321-324.

[18] Black JR, Ammerman CB, Henry PR, Littell RC. Influence of dietary manganese on tissue trace elemental accumulation and depletion in sheep. Canadian Journal of Animal Science 1985; 65: 653-658.

[19] Bremner I, Dalgarno AC. Iron metabolism in the veal calf. 2. Iron requirements and the effect of copper supplementation. British Journal of Nutrition 1973; 30: 61-76.

[20] Bremner I, Humphries WR, Phillippo M, Walker MJ, Morrice PC. Iron-induced copper deficiency in calves: dose-response relationships and interactions with molybdenum and sulphur. Animal Production 1987; 45: 403-414.

[21] Bremner I, Young BW, Mills CF. Protective effect of zinc supplementation against copper toxicosis in sheep. British Journal of Nutrition 1976; 36: 551-561.

[22] Camakaris J. Copper transport, absorption and storage. In: Howell JW, Gawthorne JM (eds.), Copper in animals and Man. Boca Raton (Flor. USA): CRC Press; 1987: 64-74.

[23] Casoli C, Duranti E, Morbidini L, Panella F, Vizioli V. Quantitative and compositional variations of Massese sheep milk by parity and stage of lactation. Small Ruminant Research 1989; 2: 47-62.

[24] Centraal Veevoeder Bureau. Tabellenboek Veevoeding. 2002.

[25] Chase CR, Beede DK, Van Horn HH, Shearer JK, Wilcox CJ, Donovan GA. Responses of lactating dairy cows to copper source, supplementation rate, and dietary antagonist (iron). Journal of Dairy Science 2000; 83: 1845-1852.

[26] Claypool DW, Adams FW, Pendell HW, Hartmann-NA J, Bone JF. Relationship between the level of copper in the blood plasma and liver of cattle. Journal of Animal Science 1975; 41: 911-914.

[26a] Commissie Bemesting Grasland en Voedergewassen. Adviesbasis bemesting grasland en voedergewassen 2002. Lelystad, The Netherlands.

[27] Commissie Onderzoek Minerale Voeding. Handleiding mineralenonderzoek bij rundvee in de praktijk. 1996.

[28] Coni E, Bocca A, Coppolelli P, Caroli S, Cavallucci C, Marinucci MT. Minor and trace element content in sheep and goat milk and dairy products. Food Chemistry 1996; 57: 253-260.

[29] Cousins RJ. Absorption, transport, and hepatic metabolism of copper and zinc: special reference to metallothionein and ceruloplasmin. Physiological Reviews 1985; 65: 238-309.

[30] Delves HT. Assessment of trace element status. Clinics in Endocrinology and Metabolism 1985; 14: 725-761.

[31] Deol HS, Howell JM, Dorling PR. Effect of the ingestion of heliotrope and copper on the concentration of zinc, selenium and molybdenum in the liver of sheep. Journal of Comparative Pathology 1994; 110: 303-307.

[32] Doyle JJ, Pfander WH. Interactions of cadmium with copper, iron, zinc, and manganese in ovine tissues. Journal of Nutrition 1975; 105: 599-606.

[33] Du Z, Hemken RW, Harmon RJ. Copper metabolism of Holstein and Jersey cows and heifers fed diets high in cupric sulfate or copper proteinate. Journal of Dairy Science 1996; 79: 1873-1880.

[34] Du Z, Hemken RW, Trammell DS. Comparison of copper tolerances between Holstein and Jersey steers. American Dairy Assocication, Oregon State University 1996; 91: So27.

[35] Dwivedi P, Swarup D, Dey S, Patra RC. Lead poisoning in cattle and buffalo near primary lead-zinc smelter in India. Veterinary and Human Toxicology 2001; 43: 93-94.

[36] Engel RW, Hardison WA, Miller RF, Price NO, Huber JT. Effect of copper intake on concentration in body tissue and on growth, reproduction and production in dairy cattle. Journal of Dairy Science 1964; 23: 1160-1163.

[37] Fick KR, Ammerman CB, Miller SM, Simpson CF, Loggins PE. Effect of dietary lead on performance, tissue mineral composition and lead absorption in sheep. Journal of Animal Science 1976; 42: 515-523.

[38] Furr AK, Parkinson TF, Heffron CL, Reid JT, Haschek WM, Gutenmann WH, Bache. Elemental content of tissues and excreta of lambs, goats, and kids fed white sweet clover growing on fly ash. Journal of Agricultural and Food Chemistry 1978; 26: 847-851.

[39] Gengelbach GP, Spears JW. Effects of dietary copper and molybdenum on copper status, cytokine production, and humoral immune response of calves. Journal of Dairy Science 1998; 81: 3286-3292.

[40] Gengelbach GP, Ward JD, Spears JW. Effect of dietary copper, iron, and molybdenum on growth and copper status of beef cows and calves. Journal of Animal Science 1994; 72: 2722-2727.

[41] Gengelbach GP, Ward JD, Spears JW, Brown TT, Jr. Effects of copper deficiency and copper deficiency coupled with high dietary iron or molybdenum on phagocytic cell function and response of calves to a respiratory disease challenge. Journal of Animal Science 1997; 75: 1112-1118.

[42] GfE. Spurenelemente. In: Staudaucher W (ed.), Empfehlungen zur Energie- und Nährstoffversorgung der Milchkühe und Aufzuchtrinder. Frankfurt am Main: DLG Verlag; 2001: 89-104.

[43] Gooneratne R, Christensen DA. Effect of chelating agents on the biliary and urinary copper excretion in sheep. TEMA 1993; 8: 1002-1005.

[44] Gooneratne SR, Christensen DA. A survey of maternal copper status and fetal tissue copper concentrations in Saskatchewan bovine. Canadian Journal of Animal Science 1989; 69: 141-150.

[45] Gooneratne SR, Symonds HW, Bailey JV, Christensen DA. Effects of dietary copper, molybdenum and sulfur on biliary copper and zinc excretion in Simmental and Angus cattle. Canadian Journal of Animal Science 1994; 74: 315-325.

[45a] Grac ND, Lee J. Effect of increasing Fe intake on the Fe and Cu content of tissues in grazing sheep. Proceedings of the New Zealand Society of Animal Production 1990; 50: 265-268.

[45b] Grace ND, Watkinson JH. Se, Cu, Zn and Fe metabolism of the young lamb. Proceedings of the New Zealand Society of Animal Production 1988; 48: 257-260.

[46] Graham TW, Thurmond MC, Mohr FC, Holmberg CA, Anderson ML, Keen CL. Relationships between maternal and fetal liver copper, iron, manganese, and zinc concentrations and fetal development in California Holstein dairy cows. Journal of Veterinary Diagnostic Investigation 1994; 6: 77-87.

[47] Groot MJ, Gruys E. Yellow discoloration in veal calves: the role of hepatic copper. Veterinary Record 1993; 132: 156-160.

[48] Grün M, Anke M, Hennig A, Seffner W, Partschefeld M, Flachowsky G, Groppel B. Überhöhte orale Eisengaben an Schafe. 2. Mitteilung. Der Einfluss auf den Eisen-, Kupfer-, Zink- und Mangangehalt verschiedener Organe. Excessive oral iron application to sheep. (2) The influence on the level of iron, copper, zinc and manganese in different organs. Archiv für Tierernährung 1978; 28: 341-347.

[49] Gueguen L, Lamand M, Meschy F. Nutrition minérale. In: Jarrige R (ed.), Alimentation des bovins, ovins & caprins. Paris: INRA; 1988: 98-111.

[50] Hapke HJ, Prigge E. Neue Aspekte der Bleivergiftung bei Wiederkäuern New aspects of lead poisoning in ruminants. Berliner und Münchener Tierärztliche Wochenschrift 1973; 86: 410-413.

[51] Harrison TJ, Ryssen JBJv, Barrowman PR. The influence of breed and dietary molybdenum on the concentration of copper in tissues of sheep. South African Journal of Animal Science 1987; 17: 104-110.

[52] Hartmans J. Hoe chronische kopervergiftiging bij schapen te voorkomen? Tijdschrift voor Diergeneeskunde 1975; 100: 405-407.

[53] Hemingway RG, MacPherson A. The accumulation of copper in the liver of housed Blackface lambs. Veterinary Record 1967; 81: 695-696.

[54] House WA, Bell AW. Mineral accretion in the fetus and adnexa during late gestation in Holstein cows. Journal of Dairy Science 1993; 76: 2999-3010.

[55] Howell JM, Deol HS, Noordin MM, Dorling PR. Trace element interactions with special reference to the interaction of copper and heliotrope in sheep. TEMA 1993; 8: 271-277.

[56] Huber JT, Price ND, Engel RW. Response of lactating dairy cows to high levels of dietary molydenum. Journal of Animal Science 1971; 32: 364-370.

[57] Huber JT, Price NO. Influence of high dietary calcium and phosphorus and Ca:P ratio on liver copper and iron stores in lactating cows. Journal of Dairy Science 1971; 54: 429-432.

[58] Humphries WR, Bremner I, Phillippo M. The influence of dietary iron on copper metabolism in the calf. In: Mills CF, Bremner I, Chesters JK (eds.), Trace elements in man and animals: TEMA 5, 5 ed. Slough: CAB; 1985: 371-374.

[59] Humphries WR, Morrice PC, Mitchell AN. Copper poisoning in Angora goats. Veterinary Record 1987; 121: 231.

[60] Humphries WR, Phillippo M, Young BW, Bremner I. Influence of dietary iron and molybdenum on copper metabolism in calves. British Journal of Nutrition 1983; 49: 77-86.

[61] Interdepartmental Working Party. Mineral, trace element and vitamin allowances for ruminant livestock. In: Haresign W, Cole DJA (eds.), Recent advances in Animal Nutrition. London: Butterworths; 1984: 113-142.

[62] Ivan M, Grieve CM. Effects of zinc, copper, and manganese supplementation of high-concentrate ration on gastrointestinal absorption of copper and manganese in Holstein calves. Journal of Dairy Science 1976; 59: 1764-1768.

[63] Ivan M, Proulx JG, Morales R, Codagnone HCV, Dayrell Md. Copper accumulation in the liver of sheep and cattle fed diets supplemented with copper sulfate or copper chloride. Canadian Journal of Animal Science 1990; 70: 727-730.

[64] Jenkins KJ. Effect of copper loading of preruminant calves on intracellular distribution of hepatic copper, zinc, iron, and molybdenum. Journal of Dairy Science 1989; 72: 2346-2350.

[65] Jenkins KJ, Hidiroglou M. Tolerance of the calf for excess copper in milk replacer. Journal of Dairy Science 1989; 72: 150-156.

[66] Jenness R, Patton S. Principles of dairy chemistry. Londen: Chapman & Hall Ltd; 1959.

[67] Jongbloed AW, Kemme PA, De Groote G, Lippens M, Meschy F. Bioavailability of major and trace minerals. Brussels: ENFEMA; International Association of the European (EU) Manufacturers of Major, Trace and Specific Mineral Materials; 2002.

[68] Jongbloed AW, Top AMvd, Beynen AC, Klis JDvd, Kemme PA, Valk H. Consequences of newly proposed maximum contents of copper and zinc in diets for cattle, pigs and poultry on animal performance and health. Report ID-Lelystad 2001; 2097.

[69] Jongbloed AW, Tsikakis P, Kogut J. Quantification of the effects of copper, molybdenum and sulphur on the copper status of cattle and sheep and inventory of these mineral contents in roughages. Lelystad: Animal Sciences Group; 2004.

[70] Kaur H, Chopra RC, Kumar V. Copper and iron metabolism in crossbred goat kids and calves. TEMA 1993; 8: 333-334.

[71] Kegley EB, Spears JW. Bioavailability of feed-grade copper sources (oxide, sulfate, or lysine) in growing cattle. Journal of Animal Science 1994; 72: 2728-2734.

[72] Kemme PA, Jongbloed AW, Mroz Z, Bruggencate Rt. Effect van het gehalte aan Ca en microbieel fytase in twee voeders op de Ca-, Mg- en P-benutting en op de beschikbaarheid van Zn en Cu bij groeiende varkens. Rapport ID-DLO 1995; 288: 1-42.

[73] Kessler J. Mineral nutrition of goats. In: Morand-Fehr P (ed.), Goat Nutrition. Wageningen: Pudoc; 1991: 104-119.

[74] Kincaid RL, Blauwiekel RM, Cronrath JD. Supplementation of copper as copper sulfate or copper proteinate for growing calves fed forages containing molybdenum. Journal of Dairy Science 1986; 69: 160-163.

[75] Kirchgessner M, Friesecke H, Koch G. Fütterung und Milchzusammensetzung. München: BLV; 1965.

[76] Kirchgessner M, Schwarz FJ, Roth HP, Schwarz WA. Interactions between the trace elements zinc, copper and iron after zinc depletion and zinc repletion of dairy cows Wechselwirkungen zwischen den Spurenelementen Zink, Kupfer und Eisen nach Zinkdepletion und -repletion von Milchkühen. Archiv für Tierernährung 1978; 28: 723-733.

[77] Klawuhn D, Staufenbiel R. Die Ermittlung der Körperzusammensetzung über die Gesamtkörperwasserbestimmung mit Phenazon zur Beschreibung des Körperfettansatzes beim Rind. 1. Mitteilung: Zusammenhang zwischen Körperfettgehalt und Lebendmasse. Estimation of body composition based on total body water analysis using phenazone for assessment of body fat gain. 1. Communication: Relationship between body fat and liveweight. Deutsche Tierärztliche Wochenschrift 1997; 104: 501-540.

[78] Koopman JJ, Wijbenga A. Het kopergehalte van levers en leverbotten bij slachtrunderen en lammeren met distomatosis (Fasciola hepatica L.). Tijdschrift voor Diergeneeskunde 1969; 94: 362-379.

[79] Lamand M. Influence of molybdenum and sulfur on copper metabolism in sheep: comparison of elemental sulfur and sulfate. Annales de Recherches Vétérinaires 1989; 20: 103-106.

[80] Lee HJ, Jones GB. Interactions of selenium, cadmium and copper in sheep. Australian Journal of Agricultural Research 1976; 27: 447-452.

[81] Ling ER. A textbook of dairy chemistry, 3rd ed. Londen: Chapman & Hall Ltd; 1956.

[82] Lopez A, Collins WF, Williams HL. Essential elements, cadmium and lead in raw and pasteurized cow and goat milk. Journal of Dairy Science 1985; 68: 1878-1886.

[83] Luit Bv, Smilde KW. Onderzoek naar de verontreiniging met cadmium en zink van grond en gewas in de omgeving van zinkfabrieken. Bedrijfsontwikkeling 1983; 14: 489-493.

[84] Luo XG, Henry PR, Ammerman CB, Madison JB. Relative bioavailability of copper in a copper-lysine complex or copper. Animal Feed Science and Technology 1996; 57: 281-289.

[85] Lüke F, Marquering B. Untersuchungen über den Mineralstoffgehalt in der Schafleber. I. Fütterungsbedingte und genetische Einflüsse aunf den Cu-Gehalt. Züchtungskunde 1972; 44: 56-65.

[86] Marcilese NA, Ammerman CB, Valsecchi RM, Dunavant BG, Davis GK. Effect of dietary molybdenum and sulfate upon urinary excretion of copper in sheep. Journal of Nutrition 1970; 100: 1399-1406.

[87] McAllister MM, Gould DH, Raisbeck MF, Cummings BA, Loneragan GH. Evaluation of ruminal sulfide concentrations and seasonal outbreaks of polioencephalomalacia in beef cattle in a feedlot. J Am Vet Med Assoc 1997.

[88] McDonald I, Mills CF, Dalgarno AC, Simpson AM. Rates of loss of hepatic copper during copper-depletion of cattle. Proceedings of the Nutrition Society 1979; 38: 59A.

[89] Miller WJ, Blackmon DM, Gentry RP, Powell GW, Perkins HF. Influence of zinc deficiency on zinc and dry matter content of ruminant tissues and on excretion of zinc. Journal of Dairy Science 1966; 49: 1446-1453.

[90] Mills CF. Biochemical and physiological indicators of mineral status in animals: copper, cobalt and zinc. Journal of Animal Science 1987; 65: 1702-1711.

[91] Mills CF, Dalgarno AC. Copper and zinc status of ewes and lambs receiving increased dietary concentrations of cadmium. Nature 1972; 239: 171-173.

[92] Milne DB. Assessment of copper nutritional status. Clinical Chemistry 1994; 40: 1479-1484.

[93] Moss BR, Madsen F, Hansard SL, Gamble CT. Maternal-fetal utilization of copper by sheep. Journal of Animal Science 1974; 38: 475-479.

[94] Nederbragt H, van den Ingh TS, Wensvoort P. Pathobiology of copper toxicity. Veterinary Quarterly 1984; 6: 179-185.

[95] NRC. Nutrient Requirements of Goats: Angora, Dairy, and Meat Goats in Temperate and Tropical Countries. Washington: National Academy Press; 1981.

[96] NRC. Nutrient Requirements of Sheep. Washington: National Academy Press; 1985.

[97] NRC. Nutrient Requirements of Beef Cattle. Washington, USA: National Academy Press; 1996.

[98] NRC. Nutrient Requirements of Dairy Cattle. Washington: National Academy Press; 2001.

[99] Oude Elferink SJWH, Meijer GAL. Sulfur-containing compounds in drinking water for cattle. (Risico's van zwavelverbindingen in drinkwater voor runderen). Report ID-Lelystad 2001; 2000.009.

[100] Ouweltjes W, Counotte GHM, Dobbelaar P. Kopervoorziening bij melkvee in West-Nederland. PraktijkRapport 4 2002.

[101] Park YW. Comparison of mineral and cholesterol composition of different commercial goat milk products manufactured in USA. Small Ruminant Research 2000; 37: 115-124.

[102] Pearl DS, Ammerman CB, Henry PR, Littell RC. Influence of dietary lead and calcium on tissue lead accumulation and depletion, lead metabolism and tissue mineral composition in sheep. Journal of Animal Science 1983; 56: 1416-1426.

[103] Pena MMO, Lee J, Thiele DJ. A delicate balance: homeostatic control of copper uptake and distribution. Journal of Nutrition 1999; 129: 1251-1260.

[104] Phillippo M, Humphries WR, Garthwaite PH. The effect of dietary molybdenum and iron on copper status and growth in cattle. Journal of Agricultural Science, UK 1987; 109: 315-320.

[105] Prabowo A, Spears JW, Goode L. Effects of dietary iron on performance and mineral utilization in lambs fed a forage-based diet. Journal of Animal Science 1988; 66: 2028-2035.

[106] Puls R. Mineral Levels in Animal Health. British Columbia Ministery of Agriculture; 1988.

[107] Rabiansky PA, McDowell LR, Velasquez-Pereira J, Wilkinson NS, Percival SS, Martin FG, Bates DB, Johnson AB, Batra TR, Salgado-Madriz E. Evaluating copper lysine and copper sulfate sources for heifers. Journal of Dairy Science 1999; 82: 2642-2650.

[108] Rodriguez-Rodriguez EM, Sanz AM, Diaz RC. Chemometric studies of several minerals in milk. Journal of Agricultural and Food Chemistry 1999; 47: 1520-1524.

[109] Sass-Kortsak A. Copper metabolism. Advances in Clinical Chemistry 1965; 8: 1-67.

[110] Saylor WW, Morrow FD, Leach-RM J. Copper- and zinc-binding proteins in sheep liver and intestine: effects of dietary levels of the metals. Journal of Nutrition 1980; 110: 460-468.

[111] Schee Wvd, Assem GHvd, Berg Rvd. Breed differences in sheep with respect to the interaction between zinc and and the accumulation of copper in the liver. Veterinary Quarterly 1983; 5: 171-174.

[112] Schonewille JT, Beynen AC. High calcium intake does not impair apparent copper absorption in goats. Journal of Animal Physiology and Animal Nutrition 1995; 73: 251-257.

[113] Schonewille JT, Yu S, Beynen AC. High iron intake depresses hepatic copper content in goats. Veterinary Quarterly 1995; 17: 14-17.

[114] Schwarz FJ, Kirchgessner M. Kupfer- und Zinkgehalte in der Milch und im Plasma von Kühen nach hoher nutritiver Kupferdosierung. Copper and zinc contents in milk and plasma of cows after high nutritional copper supplements. Zeitschrift für Lebensmittel Untersuchung und Forschung 1978; 166: 5-8.

[115] Schwarz FJ, Kirchgessner M. Zur Beifütterung hoher Kupfermengen an Milchkühe. Landwirtschaftliche Forschung 1978; 31: 317-326.

[116] Shand A, Lewis G. Chronic copper poisoning in young calves. Veterinary Record 1957; 69: 618-620.

[117] Simpson AM, Mills CF, McDonald I. Tissue copper retention or loss in young growing cattle. TEMA 1982; 4: 133-136.

[118] Smart ME, Christensen DA. The effect of cow's dietary copper intake, sire breed, age on her copper status and that of her fetus in the first ninety days of gestation. Canadian Journal of Comparative Medicine 1985; 49: 156-158.

[119] Smith BSW, Field AC, Suttle NF. Effect of intake of copper, molybdenum and sulphate on copper metabolism in the sheep. 3. Studies with radioactive copper in male castrated sheep. Journal of Comparative Pathology 1968; 78: 449-461.

[120] Sol Morales M, Palmquist DL, Weiss WP. Milk fat composition of Holstein and Jersey cows with control or depleted copper status and fed whole soybeans or tallow. Journal of Dairy Science 2000; 83: 2112-2119.

[121] Standish JF, Ammerman CB, Palmer AZ, Simpson CF. Influence of dietary iron and phosphorus on performance, tissue mineral composition and mineral absorption in steers. Journal of Animal Science 1971; 33: 171-178.

[122] Standish JF, Ammerman CB, Simpson CF, Neal FC, Palmer AZ. Influence of graded levels of dietary iron, as ferrous sulfate, on performance and tissue mineral composition of steers. Journal of Animal Science 1969; 29: 496-503.

[123] Stoszek MJ, Mika PG, Oldfield JE, Weswig PH. Influence of copper supplementation on blood and liver copper in cattle fed tall fescue or quackgrass. Journal of Animal Science 1986; 62: 263-271.

[124] Stryer L. Biochemistry, 2 ed. New York: W.H. Freeman and Company; 1981.

[125] Suttle NF. A technique for measuring the biological availability of copper to sheep, using hypocupraemic ewes. British Journal of Nutrition 1974; 32: 395-405.

[126] Suttle NF. Effects of organic and inorganic sulphur on the availability dietary copper to sheep. British Journal of Nutrition 1974; 32: 559-568.

[127] Suttle NF. Determining the copper requirements of cattle by means of an intravenous repletion technique. Trace element metabolism in man and animals 3 1978; 473-480.

[128] Suttle NF. Bovine hypocuprosis. Veterinary Annual 1983; 23: 96-103.

[129] Suttle NF. Effects of molybdenum concentration in fresh herbage, hay and semi-purified diets on the copper metabolism of sheep. Journal of Agricultural Science, UK 1983; 100: 651-656.

[130] Suttle NF. The interactions between copper, molybdenum, and sulphur in ruminant nutrition. Annual Reviews of Nutrition 1991; 11: 121-140.

[131] Suttle NF, Abrahams P, Thornton I. The role of a soil x dietary sulphur interaction in the impairment of copper absorption by ingested soil in sheep. Journal of Agricultural Science, UK 1984; 103: 81-86.

[132] Suttle NF, Abrahams PW, Thornton I. The importance of soil type and dietary sulphur in the impairment of copper absorption in sheep which ingest soil. Proceedings of the Nutrition Society 1982; 41: 83A.

[133] Suttle NF, Alloway BJ, Thornton I. An effect of soil ingestion on the utilization of dietary copper by sheep. Journal of Agricultural Science, Cambridge 1975; 84: 249-254.

[134] Suttle NF, Brebner J. A comparison of the availability of copper in copper:lysine and copper sulphate for sheep. Animal Science 1996; 62: 690.

[135] Suttle NF, McLauchlan M. Predicting the effects of dietary molybdenum and sulphur on the availability of copper to ruminants. Proceedings of the Nutrition Society 1976; 35: 22A-23A.

[136] Symonds HW, Mather DL, Hall ED. Surgical procedure for modifying the duodenum in cattle to measure bile flow and diurnal variation in biliary manganese, iron, copper, and zinc excretion. Research in Veterinary Science 1982; 32: 6-11.

[137] Todd JR. A survey of the copper status of cattle using copper oxidase (caeruloplasmin) activity of blood serum. In: Mills CF (ed.), Trace element metabolism in animals. Proceedings of WAAP/IBP International Symposium, Aberdeen, Scotland. Edinburgh: E.&S. Livingstone; 1970: 448-451.

[138] Top AMvd, Klooster ATv', Wensing T, Wentink GH, Beynen AC. leeg. Veterinary Quarterly 1995: 54-59.

[139] Top AMvd, Wensing T, Geelen MJH, Wentink GH, Klooster ATv'. Time trends of plasma lipids and enzymes synthesizing hepatic triacylglycerol during postpartum development of fatty liver in dairy cows. Journal of Dairy Science 1995; 78: 2208-2220.

[140] Underwood EJ, Suttle NF. The Mineral Nutrition of Livestock, 3rd edition ed. Wallingford: CABI; 1999.

[141] Vanderveen JE, Keener HA. Effects of molybdenum and sulfate sulfur on metabolism of copper in dairy cattle. Journal of Dairy Science 1964; 47: 1224-1230.

[142] Vos G, Teeuwen JJMH. Koper in levers van Nederlandse mestkalveren. RIKILT-Rapport 1986; 86.52: 1-18.

[143] Ward JD, Spears JW, Gengelbach GP. Differences in copper status and copper metabolism among Angus, Simmental, and Charolais cattle. Journal of Animal Science 1995; 73: 571-577.

[144] Ward JD, Spears JW, Kegley EB. Effect of copper level and source (copper lysine vs copper sulfate) on copper status, performance, and immune response in growing

steers fed diets with or without supplemental molybdenum and sulfur. Journal of Animal Science 1993; 71: 2748-2755.

[145] Ward JD, Spears JW, Kegley EB. Bioavailability of copper proteinate and copper carbonate relative to copper sulfate in cattle. Journal of Dairy Science 1996; 79: 127-132.

[146] Weiss E, Baur P. Experimentelle Untersuchungen zur chronischen Kupfervergiftung des Kalbes. Experimental studies on chronic copper poisoning in the calf. Zentralblatt für Veterinärmedizin [A] 1968; 15A: 156-184.

[147] Wensing T, Abdelrahim AI, Schotman AJH. Some aspects of extra iron supply in veal calf fattening. Veterinary Research Communications 1986; 10: 283-296.

[148] Wentink GH, Smolders G, Boxem T, Wensing T, Muller KE, Top AMvd. Lack of clinical abnormalities in dairy heifers with low blood and liver copper levels. Veterinary Record 1999; 145: 258-259.

[149] Wiener G, Herbert JG. Variation in liver and plasma copper concentrations of sheep in relation to breed and haemoglobin type. Journal of Comparative Pathology 1976; 86: 101-109.

[150] Wiener G, Suttle NF, Field AC, Herbert JG, Woolliams JA. Breed differences in copper metabolism in sheep. Journal of Agricultural Science, UK 1978; 91: 433-441.

[151] Wiener G, Wilmut I, Woolliams C, Woolliams JA, Field AC. The role of the breed of dam and of the breed of lamb in determining the copper status of the lamb. 1. Under a dietary regime low in copper. Animal Production 1984; 39: 207-217.

[152] Wittenberg KM, Devlin TJ. Effects of dietary molybdenum on productivity and metabolic parameters of lactating ewes and their offspring. Canadian Journal of Animal Science 1988; 68: 769-778.

[153] Woolliams JA, Suttle NF, Wiener G, Field AC, Woolliams C. The effect of breed of sire on the accumulation of copper in lambs, with particular reference to copper toxicity. Animal Production 1982; 35: 299-307.

[154] Woolliams JA, Wiener G, Suttle NF, Field AC. The copper content of wool in relation to breed and the concentrations of copper in the liver and plasma. Journal of Agricultural Science, UK 1983; 100: 505-507.

[155] Wouda W, Borst GH, Gruys E. Delayed swayback in goat kids, a study of 23 cases. Vet. Q. 1986; 8: 45-56.

[156] Xu C, Wensing T, Beynen AC. The effects of dietary soybean versus skim milk protein on plasma and hepatic concentrations of zinc in veal calves. Journal of Dairy Science 1997; 80: 2156-2161.

ANNEX: OVERVIEW OF THE SERIES OF CVB DOCUMENTATION REPORTS ‘REVIEWS ON THE MINERAL PROVISION IN RUMINANTS’

- CVB Documentation report Nr. 33: Reviews on the mineral provision in ruminants I: Calcium metabolism and requirements in ruminants (A.M. van den Top)
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- CVB Documentation report Nr. 44: Reviews on the mineral provision in ruminants XII: Zinc metabolism and requirements in ruminants (A.M. van den Top)
- CVB Documentation report Nr. 45: Reviews on the mineral provision in ruminants XIII: Manganese metabolism and requirements in ruminants (A.M. van den Top)
- CVB Documentation report Nr. 46: Reviews on the mineral provision in ruminants XIV: Selenium metabolism and requirements in ruminants (A.M. van den Top)
- CVB Documentation report Nr. 47: Reviews on the mineral provision in ruminants XV: Fluorine, chromium, nickel and molybdenum metabolism and requirements in ruminants (A.M. van den Top)
- CVB Documentation report Nr. 48: Reviews on the mineral provision in ruminants XVI: Contaminants: Cadmium, lead, mercury, arsenic and radio nuclides (A.M. van den Top)
- CVB Documentation report Nr. 49 (in Dutch): Literatuurstudie over de mineralenvoorziening van herkauwers XVII: Nitraat en nitriet (A.M. van den Top)
- CVB Documentation report Nr. 50 (in Dutch): Literatuurstudie over de mineralenvoorziening van herkauwers XVIII: Kwaliteit van drinkwater (A.M. van den Top)