Reviews on the mineral provision in ruminants (VIII): IRON METABOLISM AND REQUIREMENTS IN RUMINANTS

A.M. van den Top

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PREFACE

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

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

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

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

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

Utrecht/Lelystad, November 2005.

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

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

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

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Content

LIST OF ABBREVIATIONS

Abbreviation	Unit	Description
BW	kg	Body weight
DM		Dry matter
DMI	kg	Dry matter intake
Hb		Haemoglobin
Ht		Haematocrit
MCV		Mean cell volume
MCH		Mean corpuscular haemoglobin
MCHC		Mean corpuscular haemoglobin concentration
PCV		Packed cell volume
TIBC		Total iron binding capacity
UIBC		Unbound iron binding capacity

1 FUNCTIONS OF IRON IN THE BODY

The main importance of Fe is related to its role as a component of heme, which is present in haemoglobin (Hb) and myoglobin. Both Hb and myoglobin are essential for O_2 transport from the lungs to the (muscle) tissues. Haemoglobin is packaged in the erythrocytes, whereas myoglobin is found in muscle tissue. As myoglobin has a higher affinity for O_2 than does Hb, the result is an efficient transport of O_2 from the blood into the cells.
Moreover, Fe is necessary for proper function of enzymes of the electron transport chain, cytochrome oxidase, ferredoxin, myeloperoxidase, catalase, succinate dehydrogenase, and the cytochrome P-450 enzymes. Thus, Fe is involved at all stages of energy metabolism in all tissues [54;66].

2 DISTRIBUTION OF IRON IN THE BODY AND IRON KINETICS

Approximately 60% of body Fe is present as Hb [66].
The duodenum and jejunum are the primary sites of Fe absorption, where absorption is rapid. Besides this, a slow uptake occurs in the ileum [1]. Iron uptake by the intestinal cells is regulated by the Fe status of the mucosa. In rats, upregulation of Fe absorption to the maximal level took only 24 hours. Iron is more readily absorbed as Fe^{2+} (ferrous form) than as Fe^{3+} ions (ferric form) [41]. However, Fe^{3+} ions can be partly reduced to Fe^{2+} ions in the abomasum. During digestion, Fe^{2+} is usually bound to chelators such as histidine, mucine or fructose. After uptake by the mucosal cells, Fe can be transported to the basolateral membrane and bound to transferrin in the blood. If the Fe status of the body is adequate, Fe is instead bound to ferritin in the mucosal cell and excreted from the body after the death of the enterocyte. The mechanism underlying this regulation remains to be revealed.
In the blood, Fe is bound to transferrin. The TIBC represents the total capacity of the plasma to bind Fe, whereas the UIBC represents the degree of unsaturation of the plasma with Fe. Both the Cu containing ceruloplasmin and the Mo-containing xanthine dehydrogenase play a role in the transport and release of Fe, but the mechanism remains unclear.
Under normal circumstances, ferritin is the main Fe storage compound in the body. If Fe status is high, hemosiderin is the main storage form [54;66].
In the bone marrow, Fe is incorporated into protoporphyrins during the synthesis of heme in reticulocytes.

3 IRON METABOLISM

3.1 Iron absorption from different sources

3.1.1 Cattle

Bull calves (40 kg BW) fed a milk substitute ration supplemented with 30 µg Fe/g dry milk substitute powder from either $FeSO_4$, ferric citrate, Fe-EDTA or Fe phytate for 11 weeks did not show any differences in Fe or Hb metabolism between the first three sources, whereas Hb concentration and packed cell volume at the end of the experiment were lower in the phytate group [13]. Red blood cell number and mean corpuscular volume were not different between the groups.

In another experiment, 53 newborn calves (33 kg BW) were given colostrum supplemented with 20 mg Fe from either $FeSO_4$ of Fe-saturated lactoferrin [40]. The calves receiving the $FeSO_4$ treatment showed significant increases in Hb concentrations from the start of the treatment until 10 days *post partum*, whereas these values remained more or less constant in the lactoferrin-treated calves. At 10 days of age, blood Hb concentrations were ± 12.5 and 11.5 g/dL for $FeSO_4$- and lactoferrin-treated groups, respectively.

In calves (90 kg BW, 4 animals/group) fed a maize/soybean meal ration supplemented with 1000 ppm Fe from either $FeSO_4.H_2O$ or $FeCO_3$ for 28 days and dosed with ^{59}Fe, significantly higher stable Fe concentrations were found in the small intestine of the $FeSO_4$-treated group, whereas Fe concentrations in many tissues (e.g. liver, spleen, pancreas) tended to be higher in the $FeSO_4$-treated group as compared with the $FeCO_3$ treated group [44]. Small intestine Fe concentrations were 583 and 230 ppm (DM) for $FeSO_4$ and $FeCO_3$ treated groups, respectively. Concentrations of ^{59}Fe were lower in several tissues (e.g. liver, kidney, heart, pancreas and muscle) of the $FeSO_4$ treated group as compared with the $FeCO_3$ treated group. Performance, Hb, serum Fe, serum and tissue Cu concentrations, PCV and TIBC were not significantly different between the groups.

In two experiments with calves (± 200 days of age; ± 100 kg BW) on a maize/grass hay ration (72 ppm Fe (DM)) were dosed with ^{59}Fe (70-73 mg stable Fe/animal) from either ferric chloride or ferric oxide (3 animals/Fe source) [6]. In one experiment, no ^{59}Fe deposition in any tissue of the ferric oxide group could be detected, whereas in the second experiment the radioactivity levels in liver, spleen, kidney, heart and rib tissues from the ferric oxide group were significantly (3-5 x) lower than those in the ferric chloride group.

3.1.2 Sheep

In wether lambs (34 kg BW), no differences in Ht and Hb concentrations between groups fed 1600 ppm supplemental Fe from either Fe sulphate or Fe citrate could be demonstrated [60]. The Fe content of the unsupplemented maize/grass hay/soybean meal ration was not given. Iron concentrations in the liver tended to be higher and those in the spleen were significantly lower in the citrate vs. the sulphate group, respectively. Iron concentrations in kidney, heart and muscle were not different between the groups.

In an experiment with wethers (39 kg BW) the relative absorption of ferrous sulphate, ferrous carbonate (30 mg Fe each), ferric chloride (70 mg Fe) and ferric oxide (77 mg Fe) was determined (6 animals/group) [6]. The single oral Fe doses contained 150 µCi ^{59}Fe. The ration consisted of maize, cottonseed meal and grass hay. Based on tissue ^{59}Fe deposition, ferrous sulphate, ferrous carbonate and ferric chloride tended to rank in decreasing order of absorption. However, in the animals receiving ferric oxide very low Fe tissue levels were detected, which were significantly lower than those in the other groups. Selected data are given in Table 1.

Table 1 Selected data from an experiment with wethers fed different Fe sources [6]. Values are expressed as % x 10^6 of the oral dose per g of fresh tissue [a]

	Forms of Fe			
	Fe_2O_3	**$FeCO_3$**	**$FeCl_3$**	**$FeSO_4$**
Liver	25	582	464	682
Spleen	53	660	443	601
Kidney	25	326	266	355
Heart	12	87	100	97
Muscle	4	20	17	24

[a] all levels of the Fe_2O_3 group were lower than the corresponding values of the other groups

3.1.3 Goats

No data are available on differences in Fe absorption from different sources by goats.

3.1.4 Iron from drinking water

No suitable data are available on the bio-availability and the significance of Fe in drinking water. However, as Fe in drinking water is more or less dissolved, the availability is assumed to be high [54].

As explained in CVB Documentation report Nr. 41, the Fe intake from drinking water by lactating cows can be assessed to be up to ± 300 mg/day (at Fe concentrations up to 2.5 mg/L), whereas the intake from feeds will be ± 7000 mg/day. Under normal conditions, the contribution of Fe from drinking water will therefore be relatively minor. On Fe-rich (sandy) soils the Fe content of ground water can be as high as 3-8 mg/L. As such high concentrations hamper proper functioning of water pipes, taps etc., the Fe has to be removed from the water by chemical treatments. This results in Fe concentrations below the Dutch maximum tolerable lever of 2.5 mg/L (G. Counotte, Animal Health Service, personal communication).

3.1.5 Discussion and conclusions

The scarce data reported do not reveal one most suitable Fe source for use in ruminants, although – if any - $FeSO_4$ seems to be the source of first choice [53]. Higher tissue Fe deposition could be associated with lower dietary Cu concentrations (paragraph 3.2.5), which, in turn, could be associated with higher dietary sulphate levels (CVB Documentation report Nr. 41). As the use of $FeSO_4$ as an Fe source simultaneously increases the dietary sulphate content, the effect of $FeSO_4$ on tissue Fe levels could theoretically be interrelated with Cu metabolism. As to what extent this interrelationship exists remains unclear.

Iron phytate and Fe-saturated lactoferrin seem to be unsuitable Fe sources for non-ruminating calves. In ruminating animals, the specific disadvantage mentioned for Fe phytate may be irrelevant (breakdown of phytate by ruminal phytase), whereas Fe_2O_3 is an unsuitable Fe source. As Fe^{3+}ions need to be reduced to Fe^{2+}ions prior to uptake by the intestinal mucosa [70], this could be related to the poor availability of Fe_2O_3. However, as $FeCl_3$ (also containing Fe^{3+}) has been demonstrated to be well available, the insolubility of Fe_2O_3 [68] is the most important feature underlying its unsuitability as an Fe source for ruminants.

In raw materials used in concentrates, extra Fe originating from wastage of processing equipment can be present. Essentially, Fe will be present as metallic Fe or rust (Fe (hydr)oxides). Although no data are available on the availability of metallic Fe or rust for ruminants, the insolubility (metallic Fe) or very poor solubility (Fe (hydr)oxides) [68] of these sources in water most likely will make them unavailable. Thus, the extra amount of Fe added to the ration of ruminants in this way can be ignored.

3.2 Interactions influencing iron absorption

3.2.1 Iron and cadmium

3.2.1.1 Cattle

No experimental data are available on the influence of dietary Cd on Fe metabolism in cattle.

3.2.1.2 Sheep

In lambs on a diet (cottonseed hulls, maize, soybean meal, molasses) supplemented with graded levels of Cd, Fe concentrations in ileum and liver were significantly depressed in most of the Cd-treated groups when compared with control animals not receiving extra Cd [17]. Iron concentrations in rumen, abomasum, heart, spleen, lungs, testicles and kidneys were not affected. Selected results are given in Table 2.

Table 2. Selected results of dietary Cd addition on Fe metabolism of lambs [17].

Ration (ppm)			Fe (ppm (DM)	
basal Fe	**Basal Cd**	**Added Cd**	**Ileum**	**Liver**
150	0.2	0	130	369
		5	91 D	212 D
		15	86 D	326
		30	81 D	205 D
		60	76 D	180 D

D = significantly different from lowest control group receiving no additional Cd

3.2.1.3 Goats

Although dietary Cd supplements are reported to influence Fe metabolism in goats [8], no suitable data on this interaction are available.

3.2.1.4 Conclusion

In non-polluted areas, Cd levels in herbage are 0.1-0.8 ppm (DM), whereas Cd levels in polluted areas are 1-21 ppm (DM)[49]. Therefore, the results of the sheep experiment mentioned [17] are applicable in practice, which means that Fe metabolism of sheep in Cd-polluted areas might be impaired. However, due to scarcity of data quantification of the interaction is precluded.

3.2.2 Iron and zinc

3.2.2.1 Cattle

In bull calves (5-11 months of age) on a ration of hay, groundnut cake and maize, the addition of Zn (from $ZnSO_4$) gradually depressed Fe retention [11]. Retention data were 677, 529, 445 and 255 mg/day for 0, 20,40 and 60 ppm additional Zn. On the other hand, in lactating cows on a grass silage/concentrate ration supplemented with Zn (from ZnO) to contain either 44, 372, 692 or 1279 ppm Zn (DM) Hb concentrations (10.9-11.9 g/100 mL) and PCV (39.3-41.2%) were of similar magnitude in all groups [48]. Unfortunately, for none of the two experiments statistical data were given, thereby precluding judgement of the data.
In an experiment with lactating dairy cows (mean milk yield 15 kg) fed a Zn deficient, semi-synthetic diet (6 ppm Zn (DM)) for 6 weeks [38], the Fe content of the milk was 0.51 mg/kg milk. After 10 weeks Zn repletion (108-436 ppm Zn (DM)), the Fe content of the milk was slightly increased to 0.57 mg/kg milk. Corresponding serum Fe concentrations were 47 and 52 µM, respectively [38].

3.2.2.2 Sheep

In sheep (1 year of age) fed a grain/hay ration supplemented with 2667 (first 4 weeks) - 4000 ppm Zn (4-14 weeks), animals with histological kidney lesions had significantly higher kidney and pancreas Fe concentrations when compared with control animals not receiving extra Zn [5]. Iron concentrations were 14.4 vs. 5.0 µmol/g DM (kidney) and 2.0 vs. 1.5 µmol/g DM (pancreas) in Zn-supplemented and control groups, respectively.

3.2.2.3 Goats

No data are available on the influence of dietary Zn on Fe metabolism in goats.

3.2.2.4 Conclusion

As the dietary Zn concentrations mentioned to influence Fe metabolism in cattle (108-436 ppm (DM)) substantially exceed the highest recommendations for cattle (23-63 ppm Zn (DM)) [54] and probably maximum allowable concentrations (120 ppm (DM) (dairy cattle) and 100 ppm (DM) (other cattle) according to EU legislation [34]) and, nevertheless, produce only slight alterations of Fe metabolism, under practical circumstances the influence of Zn on Fe metabolism in cattle seems irrelevant. Probably the same is the case for sheep and goats, but as no suitable data are available this cannot be judged.

3.2.3 Iron and lead

Although Pb has been shown to significantly depress Hb concentrations (11.08 vs. 13.94 g/100 mL) in blood of calves after 28 days feeding of 7.5 mg Pb/kg BW (from Pb acetate)[55], no direct evidence of the influence of Pb on Fe metabolism in ruminants (if any) has been reported.

3.2.4 Iron and either nickel, aluminium, calcium and/or phosphorus

3.2.4.1 Cattle

In bull calves (50 days of age, 74 kg BW), no effect of the addition of 5 ppm Ni (from $NiCl_2.6H_2O$) to a maize/cottonseed hulls/-meal ration on Fe concentrations in liver, kidney, spleen, lung, heart or muscle has been demonstrated [59]. The experiment lasted for 140 days.

The same is the fact for the addition of 300, 600 or 1200 ppm Al (from $AlCl_3.6H_2O$) to the ration of steers (226 kg BW) [67]. The unsupplemented maize/soybean meal/cottonseed seed hulls ration contained 210 ppm Al and 3.5 g P/kg and the experiment lasted for 84 days. Iron concentrations in liver, kidney, muscle and brain were unaffected by Al treatment.

Increasing the P content of a grass hay/maize/soybean meal ration from 2.3 to 4.6 g/kg (extra P from NaH_2PO_4) during 77 days significantly decreased the Fe concentrations in liver and kidney of steers (± 200 kg BW), whereas those in spleen, heart and muscle tissue were not significantly influenced [61]. The Ca content of the ration was doubled together with the P content from 2.6 to 5.2 g/kg to maintain similar Ca:P ratios. The relative effect of increasing the P content of the ration on liver Fe content was larger when the ration contained 1000 instead of 100 ppm Fe (Fe x P interaction). Increasing the P content of the ration did not significantly influence apparent Fe absorption. Selected results are given in Table 3.

In lactating cows (mean milk yield 17.5 kg), increasing the Ca and/or P content of a maize silage/hay/concentrate ration for 12 months did not significantly influence liver Fe content [27]. Calcium was added as $CaCO_3$, whereas the combined Ca+P treatment originated from tricalcium phosphate. Selected results are given in Table 3.

Table 3. Selected results of experiments on the influence of Ca and/or P on Fe metabolism in cattle.

Ref.	Category	Ration			Fe (ppm DM)	
		Fe (ppm)	Ca (g/kg)	P (g/kg)	Liver	Kidney
[61]	Steer calves	100	2.6	2.3	273	375
			5.2	4.6	240	264
		1000	2.6	2.3	552	400
			5.2	4.6	389 D	325
[27]	Lactating cows	117	8.8	4.7	140 a	
			23	4.7	180 a	
			23	12	220 a	

D = significantly different from lowest P level within Fe level; a = values assessed from a graph

3.2.4.2 Sheep

In wether lambs (6 months of age, 31 kg BW), the influence of dietary additions of either 2.5 g P/kg, 1450 ppm Al and/or 760 ppm Fe to a basal ration (maize (starch)/cottonseed hulls; 1.7 g P/kg, 40 ppm Fe, 168 ppm Al) was investigated [58]. The duration of the experiment was 76 days. Phosphorus was added as NaH_2PO_4, Fe was added as ferric citrate and Al was added as $AlCl_3.6H_2O$. Selected results are given in Table 4. Significant P, Al and Fe effects, as well as P x Fe, P x Al, Fe x Al and P x Fe x Al interactions were observed. Iron concentrations in kidney, muscle, heart and spleen, as well as Hb concentrations and haematocrits were not influenced by Al or P supplements.

Table 4 Selected results of an experiment on the influence of dietary P, Al and Fe additions on liver Fe concentrations in sheep [58] [a].

Treatment			
P (g/kg)	Fe (ppm)	Al (ppm)	Liver Fe (ppm (DM))
0	0	0	162
2.5	0	0	164
0	760	0	1306
2.5	760	0	1091
0	0	1450	185
2.5	0	1450	188
0	760	1450	3652
2.5	760	1450	1057

[a] Significant P, Al and Fe effects, as well as P x Fe, P x Al, Fe x Al and P x Fe x Al interactions.

3.2.4.3 Goats

No data are available on any influences of dietary Ni, Al, Ca and/or P on Fe metabolism in goats.

3.2.4.4 Conclusions

Due to scarcity of data, neither Ni, Al, Ca nor P effects on Fe metabolism in ruminants can be adequately judged or quantified.

Both in cattle and sheep P effects on liver Fe concentrations depend on dietary Fe concentrations. When dietary Fe concentrations are low (40-117 ppm), increasing the P content of the ration has hardly any effect. At higher dietary Fe concentrations (800-1000 ppm), however, increasing the P content of the ration depresses the Fe content of the liver. As the Fe content of grass silage can easily attain or exceed 800 ppm Fe (both intrinsic Fe and Fe from rain soil splash (CVB Documentation report Nr. 41)) the influence of higher dietary P concentrations on Fe metabolism should be taken into account. However, Fe requirements are in the order of magnitude not influenced by P additions, whereas high P

contents of ruminant rations are not likely to occur in the light of restrictions of P fertilization levels.

3.2.5 Iron and copper

3.2.5.1 Cattle

In three experiments with bull calves (37-40 kg BW) [12;13;31] on a milk substitute ration, the influence of Cu additions (from $CuSO_4$) on Fe metabolism was investigated. Selected results are given in Table 5. The addition of ± 11.4 ppm Cu during 4 weeks decreased the liver non-heme Fe concentrations and transiently increased plasma Fe concentrations, whereas liver total Fe, kidney, spleen and muscle Fe concentrations were not different [13]. In a similar experiment by the same authors [12] the addition of 5 ppm Cu (DM) to the ration of calves significantly lowered Fe concentrations in spleen and heart tissues, whereas parameters of Hb (Hb, MCV, MCH, MCHC) and Fe metabolism (plasma Fe, transferrin saturation, liver (non-heme) Fe, liver ferritin, liver hemosiderin) were not influenced. In the third experiment [31], the addition of either 10 or 1000 ppm Cu (DM) significantly increased liver Fe concentrations. Within the hepatocytes, the increases were mainly due to Fe loading of the nuclei.

Table 5 Selected results of experiments on the influence of Cu on Fe metabolism in calves.

Ref.	Ration		Fe				Cu
	Fe	**Cu**	**liver**	**liver (non-heme)**	**spleen**	**heart**	**liver**
	ppm in milk powder		**ppm (DM)**				
[13]	9.8	0.8	56.7	23.7	258		25
		12.2	53.7	15.4 D	318		452 D
[12]	10	0.5	125	37.8	850	234	69
	40		83.5	30.4	936	185	54
	100		103	48.8	1191	234	64
	10	5.5	57	22.9	433	133	562
	40		88	23.5	568	183	556
	100		108	28.1	699 D	227 D	586
[31]	109 (DM)	10 (DM)	97				
		1000 (DM)	130				

D = significant Cu effect.

In calves fed whole and skim milk containing either 2 or 10 ppm Cu (DM), no difference could be observed in performance or meat colour [18]. In lactating dairy cows on a ration (maize silage and -meal, lucerne hay, cottonseed) with or without additional Cu (0, 15 or 30 ppm Cu (DM)) from either $CuSO_4$ or Cu-Lysine no effect of Cu addition on liver Fe concentrations could be observed [15].

3.2.5.2 Sheep

In sheep (11 kg BW) on a ration (maize, rice bran, groundnut cake, palm kernel meal) significant influences of Cu addition (5 ppm) on Fe metabolism could be observed [2]. Iron was added as $FeSO_4.7H_2O$ and Cu as $CuSO_4.5H_2O$. Selected results are given in Table 6.

Table 6 Selected results of an experiment on the influence of Cu on Fe metabolism in sheep [2].

Ration		Plasma	Liver		Heart	Ht	Hb	CP
Fe	Cu	Fe	Cu	Fe				
ppm		µM	ppm (DM)			%	g/dL	IU
20	4	20.3	198	169	128	29.3	9.3	38.2
35	4	24.5	196	361	139	30.6	9.9	41.3
20	9	22.4	822 D	143	114	31.3	12.9 D	45.8 D
35	9	30.8 D	769 D	248 D	121	36.8 D	14.6 D	47.3 D

Ht = haematocrit; Hb = haemoglobin concentration; CP = caeruloplasmin activity; D = significant Cu effect within Fe treatment.

3.2.5.3 Goats

As yet, no data are available on the influence of dietary Cu on Fe metabolism in goats.

3.2.5.4 Conclusion

Regarding the high dietary Cu levels employed in the third calf experiment [31], these Cu effects may be rather pharmacological than physiological. Thus, results of this experiment have been ignored. Although dietary Cu supplementation in cattle seems to decrease tissue Fe concentrations in favor of plasma Fe concentrations, the evidence is not identical. In sheep, the only experiment available demonstrates this phenomenon more clearly. However, due to scarcity of data quantification of the relationship is difficult.

3.2.6 Iron and manganese

3.2.6.1 Sheep

In milk-fed lambs the influence of increasing amounts of Mn on Fe metabolism was investigated [24]. During the experimental period of 35 weeks, lambs were fed either 0, 15-2500 or 45-5000 ppm Mn. Serum Fe and Hb concentrations were significantly lower in the highest Mn group compared to in the other groups. Final liver, kidney and heart Fe concentrations were not significantly different between the unsupplemented and the highest Mn-supplemented group, whereas spleen Fe concentrations were significantly lower in the highest Mn-supplemented group (204 ppm (DM)) than in the unsupplemented group (841 ppm (DM)). In a second experiment, lambs were fed a soybean hay/glucose/casein/cottonseed oil diet either not supplemented with Mn or supplemented with 1000 or 2000 ppm Mn. Both Mn-supplements resulted in significantly lower Hb and serum Fe concentrations.

3.2.6.2 Cattle and goats

No data are available on any interaction of Mn with Fe in cattle or goats.

3.2.6.3 Conclusion

As adverse effects on Fe metabolism were observed in the lambs from the 45-5000 ppm Mn group, which is well beyond Mn requirements (20 ppm (DM)) or even exceeds the recommended maximum tolerable level [52], practical value of the results mentioned is very limited. Within practical dietary Mn levels, no adverse effect of Mn on Fe metabolism should be expected.

3.3 Recycling

The Fe of senescent or defective red cells is broken down and taken up by transferrin or ceruloplasmin or stored as ferritin [1]. No data are available on enterohepatic recycling of Fe in ruminants.

3.4 Excretion

When compared with faecal Fe excretion (281-485 ppm), urinary Fe excretion (0.72-1.52 µg/mL) in calves is negligible [11]. This was also demonstrated in another experiment with young calves, where 84.5% of daily Fe intake was excreted via the faeces, whereas only 0.3% was excreted via the urine [35]. The same is the case in sheep, as within 7 days after dosing ^{59}Fe ± 90% of dose was excreted via the faeces, whereas only 0.05-0.09% of dose was excreted via the urine [6]. In another experiment with sheep, urinary Fe excretion was 1.0% of total daily Fe excretion [39]. However, in an experiment with goat kids, urinary Fe excretion was relatively high (11.5% of daily intake), while 45.2% was excreted via the faeces [35].
In calves on a hay/concentrate ration, Fe intake was 655 mg/day [65]. Corresponding daily biliary Fe excretion was 2.4 mg (0.4% of intake). Biliary Fe excretion increased substantially (285-993 µmol/day) after infection with *Fasciola hepatica*. In sheep, biliary Fe excretion did not respond to intravenous administration of tetrathiomolybdate (TM) (0.36 vs. 0.28 mg/day for groups receiving 0 and 100 mg TM, respectively [22]. No data are available on Fe losses via integument and sweat in ruminants.

4 IRON REQUIREMENTS

4.1 Cattle

Iron requirements depend on the criterion of adequacy used. Only very few data are available on true Fe absorption in ruminants. In calves fed a liquid diet, the true Fe availability decreases from 40 to 15% as the Fe content of the diet raised from 40 to 100 mg/kg [9]. In pregnant ewes, a true availability of 21% has been reported, whereas for adult ruminants a value of 10% has been suggested [54]. In this report, for calculation of Fe requirements of adult animals a value of 10%, and for milk-fed, growing animals a mean value of 28% was used.

4.1.1 Dairy cattle

Due to very effective recovery of Fe incorporated in tissues, maintenance requirements for Fe are negligible [54].
Iron content of growing tissues is reported to be 28-34 [9] (mean 31) or 18-34 mg/kg growth [54] (mean 26) and declines with age [66]. However, 13-18 mg/kg have been demonstrated to be associated with growth retardation [9]. On the other hand, a higher estimate of 55 mg/kg growth, allowing for tissue Fe storage, has been made [64]. To calculate minimum requirements, the latter value has been ignored and the average of 26 and 31, i.e. a value of 28.5 mg Fe/kg growth is assumed.
In veal calves intended to produce white meat, Fe supply is critical. At birth, the Fe status of the calves is very variable [47], thereby influencing Fe absorption. Usually, a higher Fe level (60-150 mg Fe/kg) is fed during the first 7 weeks of the fattening period, whereas 8-17 mg Fe/kg is fed until the end of the fattening period at 26-28 weeks of age to produce the desired pale meat colour. No differences in Hb concentrations at slaughter between groups fed 60, 100 of 150 mg Fe/kg of milk replacer during the first 7 weeks could be observed, as all calves became anaemic. An extra Fe supply in the middle of the fattening period might be more effective [50].
In pregnant cows consuming a ration containing 261 ppm Fe (DM), the gravid uterus accumulates approximately 18.0 mg of Fe/day [26] or 27.3 mg Fe/day [20] from 190 days of gestation until calving. No more data are available on Fe requirements during other stages of pregnancy. The average of these two values, i.e. 23 mg, has been used for factorial estimation.
The Fe content of mature milk is assessed to be 0.2 [30], 0.30 [3;33], 0.5 [37;66], 0.515 [57], 0.51-0.57 [38], 1 [54] or 1-2 mg/kg [42]. Arbitrarily, a value of 0.5 mg/kg milk has been chosen for all ruminants. An abberating high value of 2.84 mg/kg [43] has been ignored.

4.1.2 Beef cattle

No separate calculations are recommended for the Fe requirements of beef cattle as compared to dairy cattle [53].

4.2 Sheep

Calculation of the Fe requirements of sheep is essentially similar to that of cattle. Endogenous loss is similar to that of cattle. For Fe accumulation into the gravid uterus, the value for cows related to metabolic body weight ($kg^{0.75}$) is used. Thus, for a 75 kg ewe (25 $kg^{0.75}$) instead of a 650 kg cow (128 $kg^{0.75}$) the Fe accretion in the gravid uterus should be 3.6 mg/day. For ewes carrying 2 foetuses instead of 1 foetus, this value is multiplied by the same factor as for the energy need (1.7 [19]). For twin pregnancy, the Fe accretion should be 6.1

mg/day. Unless proven otherwise, for the remaining parameters the values for cattle are used.
Sheep milk is reported to contain (mg/kg) 0.38-0.40 (no differences between parities) [14] or 0.75 mg Fe/kg.
No data are available on Fe requirements for gestation or wool production in sheep.

4.3 Goats

For goats, data on Fe content of the gravid uterus, growing tissues and hair are lacking. Unless proven otherwise, the values for sheep are used.
Mature goat milk contains (mg/kg) 0.30 [16], 0.45 [46], 0.520 [57] or 0.55. An abberating high value of 6.28 mg/kg [43] has been ignored.

4.4 Conclusion

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

$$C = \frac{100 \times ((\text{kg milk} \times 0.5) + (\text{kg growth} \times 28.5) + a)}{A_{Fe} \times \text{DMI}}$$

in which
- C = required dietary Fe concentration (ppm (DM))
- A_{Fe} = true Fe absorption (%); for calculation of requirements, a value of 10% (adult) and 28% (milk-fed, growing animals) is assumed
- DMI = dry matter intake (kg/day)
- a = amount of Fe needed for gestation (23 mg/day for cattle during the last trimester); for sheep and goats, the amount of Fe needed for gestation is roughly estimated to be 3.6 or 6.1 mg/day for 1 vs. 2 foetuses, respectively (see text).

Note: endogenous Fe loss is considered to be negligible

5 ALLOWANCES

Using the equation mentioned (paragraph 4.4), some examples of dietary Fe requirements and allowances have been tabulated (Table 7). These allowances include a safety margin of 50%.

Table 7 Examples of calculated Fe requirements and allowances.

Category	DMI (kg)	Requirement (mg/day)	Allowance	
			mg/day	Ppm (DM)
Growing female cattle				
4 months, 850 g growth/day, 130 kg BW	3.9	242	363	93
9 months, 700 g growth/day, 250 kg BW	5.6	299	299	53
16 months, 625 g growth/day, 400 kg BW	7.3	267	267	37
Dairy cattle (650 kg BW)				
Cow, dry, pregnant (last trimester)	11.3	230	345	31
Cow, lactating, 20 kg of milk	18.5	100	150	8
Cow, lactating, 40 kg of milk	23.5	200	300	13
Beef cattle, intermediate type				
1000 g growth/day, 100 kg BW	3	285	428	143
1200 g growth/day, 250 kg BW	6	342	513	86
1100 g growth/day, 500 kg BW	9	314	470	52
Veal calves				
1150 g growth/day, 150 kg BW	4.5	328	492	109
1400 g growth/day, 275 kg BW	7	413	620	89
Sheep (75 kg BW)				
Growing lamb, 0.3 kg growth/day, 40 kg BW	1.6	86	128	80
Sheep, pregnant, last trimester	1.9	36	53	28
Sheep, lactating, 3 kg of milk, nursing 2 lambs	2.6	15	23	9
Goats (70 kg BW)				
Goat, pregnant, last trimester	1.7	36	53	31
Goat, lactating, 4 kg of milk	3.2	20	30	9

The allowances calculated here are in part substantially lower than most other recommendations. For preruminant calves, growing cattle (>150 kg BW) and sheep the IDWP recommends 30 ppm (DM), and for weaned calves, pregnant and lactating cattle 40 ppm (DM) [29]. In beef cattle, 40-50 ppm Fe is considered adequate to support growth (no data given) and to prevent anaemia. For calves <150 kg BW and for pregnant cattle, the ARC recommends 40 ppm (DM) [9]. The NRC recommends 50 ppm Fe for beef cattle [53]. For sheep, recommendations are 40 ppm (25 ppm being not sufficient to support maximum growth of lambs) [54] or 30 ppm (DM) [9], whereas (without specific results for goats) 30-40 ppm (DM) Fe is supposed sufficient for this animal species [4;36]. As most references do not supply any data on the calculation of their recommendations, proper comparison is precluded.

For young, growing animals, Fe requirements are relatively high (i.a. due to Fe needs for Hb synthesis). The ARC calculates an Fe requirement of 100 ppm (DM) for calves <150 kg BW, as 40 ppm (DM) is considered adequate for growth and 100 ppm (DM) is considered adequate for both growth, Hb and myoglobin synthesis. Moreover, the NRC calculates a Fe requirement of 150 ppm (DM) for a 6-week calf. These recommendations are more or less similar to those calculated above. For young dairy calves and veal calves, IDWP recommendations may not be fully adequate.

6 CRITERIA TO JUDGE IRON STATUS

6.1 Criteria to judge iron status

6.1.1 Blood parameters of iron status

When Fe deficiency is suspected, Fe status is initially assessed by determining blood Hb concentrations and Ht (PCV) values. Under normal circumstances, these parameters are highly correlated. Normal values for Hb concentrations and Ht are given in Table 8. However, individual variation is considerable and low values are not always associated with Fe deficiency. By dividing the Hb reading by the PCV, the mean cell haemoglobin concentration (MCHC) can be calculated. In cases of Fe deficiency anaemia, the MCHC is lowered due to the release of small, new erythrocytes containing less than normal Hb. Meanwhile, TIBC and UIBC increase due to an increased capacity of the blood to bind Fe (TIBC), which cannot be satisfied (UIBC). The diagnostic value of serum ferritin is limited because concentrations become minimal before anaemia develops [66].

More detailed surveys giving normal values (TIBC, UIBC) or marginal bands (indicating for deficient or excessive Fe supply; liver and kidney Fe concentrations, serum ferritin concentrations) have been given in reference [56] and [66] (Tables 1 and 2).

Table 8 Normal values for Hb and Fe concentrations and Ht values.

Category	Hb (g/L)	Serum Fe (µM)	Serum Fe marginal bands (µM)		Ht (%)	Reference
			Deficiency	Excess (E)/toxicity (T)		
Cattle	90-140	17.9-35.7	2.7-23.2	321-446 (T)	40-60	[56]
	110-120	17.4 ± 5.2	8.9-17.9	10.7-32.2 (E)		[66]
	80-150				24-46	[7]
Veal calves	80-100 [a]					[25]
	90					[69]
Cattle	5.0-8.0 [b]	27-45			27-36	[63]
Sheep	80-160	29.6-39.6			23-48	[56]
	100-110	34.6 ± 1.3	<29	>39 (E)		[66]
	90-140					[c]
	6.3-7.7 [b]	22-47			31-37	[63]
Goats	80-140	8.9-17.9			15-30	[56]
	100-110					[66]
	80-120				22-38	[7]
	80-120					[c]
	4.7-7.6 [b]				19-32	[63]

[a] the lower limit to be used for individual calves and the upper limit as a group mean value;
[b] values expressed as mM;
[c] Kessels, Utrecht University, personal communication

6.2 Conclusions

Both Hb and Ht are cheap, convenient methods to determine Fe status in most cases, although low values are not always indicative for Fe deficiency. Especially in young animals, one should be aware of (parasitic) infections causing low Fe status not related to a deficient dietary Fe supply. For use in ruminants, regarding the rare occurrence of Fe deficiency and the usually abundant Fe supply, the use of more refined methods to discriminate between the different causes of anaemia will be rarely useful. On the other hand, Fe overload can be manifested as Cu deficiency symptoms, especially when Mo concentrations of the ration are low (CVB Documentation report Nr. 41).

7 DEFICIENCY

The most striking feature of Fe deficiency is hypochromic microcytic anaemia due to insufficient Hb production. In veal calves, anaemia and a light meat colour (low myoglobin levels) is elicited by restricted Fe feeding. Moreover, poor appetite and growth, as well as an increased disease incidence due to impairment of immune function may occur. This feature can be observed before effects on red blood cell volume are detectable [54]. In veal calf production, the occurrence of anaemia does not preclude rapid growth, demonstrating the tolerance of these animals to Fe-induced anaemia. In case of twins, anaemia is more likely to occur due to the competition of the two foetuses for the limited Fe supply by the dam [66].
In adult animals, Fe deficiency is very rare due to efficient adaptation of Fe metabolism in case of an insufficient Fe intake and to the ubiquitous occurrence of Fe in the environment (e.g. soil) [54]. However, anaemia due to bloodsucking parasites is possible both in growing and adult animals [66]. Dietary Fe concentrations below 40 [56], 40-60 (cattle) or 30-50 ppm (DM) (sheep) are considered to indicate for a deficient Fe supply [66] (Table 2). However, it is not clear if this applies only to young, growing animals or also to adult ones.

7.1 Direct measures in deficiency cases

7.1.1 Direct continuous supplementation

Daily oral supplementation of the ration with 30-60 mg Fe/day or 40 mg Fe/kg DM (e.g. from $FeSO_4$) has been shown to be effective to treat Fe deficiency in newborn calves [66].

7.1.2 Direct discontinuous supplementation

Both in the neonatal lamb (200 mg Fe) and calf (500 mg Fe) iron dextran can be applied intramuscularly to treat Fe deficiency [66].

7.1.3 Slow release oral supplementation and indirect supply via fertilization

Because of the rare occurrence of Fe deficiency in adult cattle and the fact that Fe occurs ubiquitously in the environment, both slow release oral supplementation and indirect Fe supply via fertilization of the pasture are irrelevant [54].

8 TOXICITY

Free Fe is cytotoxic because of its ability to generate free oxygen radicals, which can cause e.g. membrane damage. Therefore, the extent to which tissues are affected depends on the animal's antioxidant status (vitamin A and E, carotene, Se, Cu, Zn). Moreover, a high intake of polyunsaturated fatty acids (spring grass) from Fe-rich soils may present a hazard. Finally, plants such as lupines and brassicas may increase the hepatic Fe content [54;66].
Veal calves have been demonstrated to be very tolerant for excess Fe in their milk replacer [32]. Three-day-old calves were fed a milk substitute ration supplemented with Fe (from $FeSO_4.7H_2O$) for 6 weeks. Total Fe concentrations were either 100, 500, 1000, 2000 or 5000 ppm (DM). The animals tolerated all except the 5000 ppm level. At this level, DMI (0.85 vs. 0.96-1.02 kg/day) and growth rate (0.51 vs. 0.62-0.69 kg/day) were significantly reduced when compared with the other groups. No more clinical abnormalities or gross post mortem changes were observed. Similar clinical observations were made in steers receiving Fe from $FeSO_4$ (either 100 or 1000 ppm total dietary Fe [61] or either 77, 477 or 1677 ppm Fe [62]). However, growth rate was already significantly reduced at the 1000 ppm level when compared with the 100 ppm level [61]. Similarly, the 1677 ppm as compared with the 477 ppm Fe treatment diminished DMI and growth rate, whereas the feed/gain ratio was higher [62]. A similar treatment using 3277 ppm total Fe had to be interrupted due to continuous weight loss. As these experiments were carried out using ruminating animals, the influence of S from the sulphate reducing Cu status (significantly lower liver and kidney Cu concentrations) may have contributed to the observed growth depression. However, in an experiment with calves (90 kg BW, 12 weeks of age), no effect on performance or Hb metabolism of 1000 ppm Fe from either ferrous carbonate or ferrous sulphate when compared with a 100 ppm Fe ration could be observed [44].
In summary, although symptoms of Fe toxicity are relatively mild, dietary Fe concentrations within those occurring in practical forages (2000-4000 ppm (DM) [28]) have been demonstrated to impair animal performance. For dairy cattle feeds, maximum tolerable levels of 1000 [54] or even 1000-4000 ppm Fe (DM) [56;66] have been recommended. For beef cattle, a level of 1000 ppm Fe [53], and for sheep levels of 500 [52] or 600-1200 ppm Fe [66] have been recommended.

8.1 Direct measures in toxicity cases

There is no measure reported to be effective in curing Fe toxicosis. The body has only limited possibilities to remove excess Fe [45].

9 PREVENTION

As free Fe is very reactive and generates free radicals [54;66], improving the antioxidant status of the animal (e.g. vitamin E, Se) may be helpful in overcoming Fe toxicosis. No specific measure is known to prevent Fe toxicosis.

Table 9 Inventory of Fe allowances for cattle, sheep and goats as used in some foreign countries (ppm (DM)).

		Allowance				
Country	**Ref.**	**Cattle**	**Ref.**	**Sheep**	**Ref.**	**Goat**
Great Britain	[9]	100 (growth) 30 (mature)	[9]	30	[4]	30-40
USA[a,b]	[53;54]	150 (6-week calf) 24 (lactation,gestation) 50 (beef cattle)	[52]	30	[51]	300
Germany	[21]	50		?	[10]	40-50
France	[23]	?				

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

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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)
- CVB Documentation report Nr. 34: Reviews on the mineral provision in ruminants II: Phosphorous metabolism and requirements in ruminants (H. Valk)
- CVB Documentation report Nr. 35: Reviews on the mineral provision in ruminants III: Magnesium metabolism and requirements in ruminants (J.Th. Schonewille and A.C. Beynen)
- CVB Documentation report Nr. 36: Reviews on the mineral provision in ruminants IV: Sodium metabolism and requirements in ruminants (J.Th. Schonewille and A.C. Beynen)
- CVB Documentation report Nr. 37: Reviews on the mineral provision in ruminants V: Potassium metabolism and requirements in ruminants (J.Th. Schonewille and A.C. Beynen)
- CVB Documentation report Nr. 38: Reviews on the mineral provision in ruminants VI: Chlorine metabolism and requirements in ruminants (J.Th. Schonewille and A.C. Beynen)
- CVB Documentation report Nr. 39: Reviews on the mineral provision in ruminants VII: Cation Anion Difference in Dairy Cows (J.Th. Schonewille and A.C. Beynen)
- CVB Documentation report Nr. 40: Reviews on the mineral provision in ruminants VIII: Iron metabolism and requirements in ruminants (A.M. van den Top)
- CVB Documentation report Nr. 41: Reviews on the mineral provision in ruminants IX: Copper metabolism and requirements in ruminants (A.M. van den Top)
- CVB Documentation report Nr. 42: Reviews on the mineral provision in ruminants X: Cobalt metabolism and requirements in ruminants (A.M. van den Top)
- CVB Documentation report Nr. 43: Reviews on the mineral provision in ruminants XI: Iodine metabolism and requirements in ruminants (A.M. van den Top)
- 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)