Overview: Seven trace elements in Icelandic forage. Their value in animal health and with special relation to scrapie

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ABSTRACT

The dividing line between *trace elements* (*microelements*) and *macroelements* is tentatively defined, as well as the so-called *critical amounts* or *concentrations* of the trace elements.

This review is mainly based on analyses of Mn, Cu, Mo, Se, Co, Zn and Fe in samples of forage in Iceland from the summer harvests in 2001-2003, mostly of grass silage (30-70% dry matter). The forage samples were taken on farms in various scrapie categories. Notes are given on the occurrence of the seven trace elements in Icelandic rock and soils, as well as on their mechanisms of action and essentiality and toxicity in humans. The results of Co and Zn analyses have not been published before. The results are discussed in terms of essentiality and toxicity to plants and domestic animals, especially cattle and sheep, and with special relation to the occurrence of clinical scrapie in Iceland.

The main results were as follows. *Manganese* concentrations in feed varied more than tenfold (40-550 mg kg^{-1}). Cases of Mn deficiency in cattle, sheep or poultry are not known to occur in Iceland. The mean

Mn concentration was significantly higher in samples from scrapie-free farms than from scrapie-prone or scrapie afflicted farms.

Copper concentrations varied fourfold (4-16 mg kg^{-1}). There is no evidence of Cu deficiency in plants or sheep in Iceland at the present time. The mean Cu concentration was statistically the same in samples from farms in all scrapie categories. The Mn/Cu ratio was found to be significantly higher in samples from scrapie-free farms than from scrapie-afflicted farms.

Molybdenum concentrations varied enormously (0.0043-2.37 mg kg^{-1}) and the Cu/Mo ratio was always in excess of 4. The Mo amounts available may be insufficient for the cultivation of certain plants and the Mo concentrations in forage of sheep were usually in the low normal range. The Mo concentrations did not differ significantly between farms in the various scrapie categories.

Selenium concentrations varied about fifteenfold (6-96 μg kg^{-1}). Symptoms of Se deficiency (white muscle disease) occur ubiquitously in lambs, and to some extent in heifers and foals, in spite of prophylactic use of injections of selenite and tocopherol (vitamin E). When the ewes are rounded up and gathered from the highlands in the autumn the Se levels in blood are sufficient but border on deficiency when they are in the pregnant state in the following spring after they have been kept in sheds for months and fed on forage from the cultivated home fields. The mean values did not differ significantly between farms in the various scrapie categories.

Cobalt concentrations varied fiftyfold (41-2010 μg kg^{-1}). In about 12% of the samples the Co concentration was less than 100 μg kg^{-1}. The mean concentrations did not differ significantly between farms in the various categories.

Zinc concentrations were in the range of 14-85 mg kg^{-1}. In only a few samples (7%) did the Zn concentration exceed 50 mg kg^{-1}, indicating that the Zn load in sheep might be rather low. A symptom of Zn deficiency, parakeratosis in cattle and sheep, has been observed a few times in Iceland. The mean Zn concentration was significantly higher in forage samples from scrapie-free farms in scrapie-free areas than in samples from farms in the other categories.

Iron concentrations varied almost twenty-fivefold (57-1379 mg kg^{-1}). The mean Fe concentration in samples from scrapie-free and scrapie-prone farms was significantly lower than in scrapie-afflicted farms where it was in excess of 300 mg kg^{-1} . The high Fe concentration in samples from scrapie-afflicted farms bordered on toxic levels for iron in plants and was reciprocated in the low Mn concentration found in samples from these farms.

It was concluded from this research that: 1) the low levels of Se in the forage should be amended by general measures, e.g. by use of Se fertilizers; 2) the levels of Mo, Co and Zn in forage of sheep may on occasion be too low and the same might also apply to Mn and Mo for the cultivation of certain plants; 3) the Cu status of plants and sheep is considered sufficient; 4) the highest amounts of Fe in forage samples from scrapie-afflicted farms may border on toxic levels for plants; 5) the Fe concentration was lowest in forage from scrapie-free farms whereas the opposite was the case for Mn (and to some extent for Zn); 6) high Mn concentration in forage from scrapie-free farms may indicate a protective impact on the occurrence of clinical scrapie; 7) high Fe concentration in forage from scrapie-afflicted farms may, due to suppression of Mn, be provocative of the disease; 8) detailed studies on Fe and Mn (and preferably aligned with studies on Cu and Zn) in the soil and forage on sheep farms might contribute towards a better understanding of why scrapie is repeatedly found on certain sheep farms but not on other farms, even not on those in close proximity to the afflicted farms.

Keywords: trace elements, forage, animal health, scrapie, Fe/Mn ratio

YFIRLIT

Yfirlitsgrein: Sjö snefilefni í íslensku heyi og gildi þeirra fyrir heilbrigði búfjár með sérstöku tilliti til sauðfjárriðu

Í inngangi er stutt skilgreining á *snefilmörkum (trace limits)*, sem eru skilin milli *smáefna (microelements)* eða *snefilefna (trace elements)* og *stórefna (macroelements)*, svo og á *þarfamagni* snefilefna *(critical amounts/critical concentrations*).

Samantektin byggir að mestu á ákvörðunum á Mn, Cu, Mo, Se, Co, Zn og Fe í heysýnum sem safnað var af uppskeru áranna 2001-2003. Flest sýnin voru af rúlluheyi (30-70% þurrefni). Sýnunum

var safnað á sauðfjárbúum í mismunandi riðuflokkum. Vikið er að dreifingu þessara snefilefna í íslensku bergi og jarðvegi, verkunarháttum þeirra og fjallað um hversu nauðsynleg eða eitruð þau eru fyrir menn. Niðurstöður ákvarðana á Co og Zn hafa ekki verið birtar áður. Niðurstöðurnar eru rædd- ar í ljósi þess hversu nauðsynleg efnin eru fyrir plöntur og dýr, einkum nautgripi og sauðfé, og hvort þau geti valdið eitrun. Tengslin við uppkomu klínískarar riðu eru sérstaklega skoðuð.

Helstu niðurstöður þessara rannsókna voru:

Mangan. Meira en tífaldur munur var á magni Mn í heyinu (40-550 mg kg^{-1}). Ekki er vitað um mangans-kort í nautgripum, sauðfé eða alifuglum á Íslandi. Meðalmagn Mn var marktækt meira í sýnum frá riðulausum bæjum en frá riðubæjum eða fjárskiptabæjum.

Kopar. Fjórfaldur munur var á magni Cu í sýnunum (4-16 mg kg^{-1}). Ekki er vitað um koparskort í jurtum eða sauðfé á Íslandi nú. Ekki var marktækur munur á meðalmagni Cu í sýnum frá bæjum í mismunandi riðu-flokkum. Mn/Cu-hlutfallið var marktækt hærra í sýnum frá riðulausum bæjum en frá riðubæjum.

Mólýbden. Mjög mikill munur var á magni Mo í sýnunum (0,0043-2,37 mg kg^{-1}). Cu/Mo hlutfallið var alltaf hærra en 4. Aðgengilegt magn Mo kann að vera of lítið við ræktun vissra plantna og Mo í heyi sauðfjár var oftast nokkuð í minna lagi. Ekki var marktækur munur í sýnum frá bæjum í mismunandi riðuflokkum.

Selen. Um fimmtánfaldur munur var á magni selens í sýnunum (6-96 µg kg^{-1}). Einkenni selenskorts (hvítvöðvaveiki) eru vel þekkt í lömbum, og að einhverju marki í kvígum og folöldum, þrátt fyrir töluverða varnandi notkun seleníts og tókóferóls (E vítamíns) í formi stungulyfja. Þegar ær koma af fjalli að hausti er selenþéttni í blóði þeirra nægjanleg, en þéttnin er við skortsmörk í þeim lembdum að vori eftir að ærnar hafa verið hýstar mánuðum saman og fóðraðar á heyi af ræktuðum túnum. Ekki var marktækur munur á meðalgild-um milli bæja í mismunandi riðuflokkum.

Kóbalt. Um fimmtíufaldur munur var á magni kóbalts í sýnunum (41-2010 µg kg^{-1}). Í um 12% sýnanna var Co magnið minna en 100 µg kg^{-1}. Ekki var marktækur munur á Co magni milli bæja í mismunandi riðu-flokkum.

Sink. Magn sinks var á bilinu 14-85 mg kg^{-1}. Aðeins í fáum sýnanna (7%) var magnið meira en 50 mg kg^{-1}, sem bendir til þess, að sinkbirgðir gætu verið í minna lagi í íslensku fé. Vitað er til þess að einkenni um sink-skort (parakeratosis) hafi sést í nautgripum og sauðfé á Íslandi. Sinkmagnið var marktækt meira í heysýnum frá riðulausum bæjum á riðulausum svæðum en í sýnum frá bæjum í öðrum riðuflokkum.

Járn. Næstum tuttuguogfimmfaldur munur var á magni Fe í sýnunum (57-1379 mg kg^{-1}). Meðalmagn járns í sýnum frá riðulausum bæjum og fjárskiptabæjum var marktækt minna en í sýnum frá riðubæjum þar sem það var meira en 300 mg kg^{-1} . Hið mikla járnmagn í sýnum frá riðubæjum er á mörkum eitrunar fyrir plöntur og er samfara litlu magni Mn í sýnunum frá þessum bæjum.

Af þessum rannsóknum má draga eftirfarandi ályktanir: 1) auka þarf magn Se í fóðri sauðfjár, t.d. með notkun selenáburðar; 2) magn Mo, Co og Zn í heyi sauðfjár kann að vera of lítið í sumum tilfellum, og hið sama gæti einnig átt við um Mn og Mo fyrir ræktun sumra nytjaplantna; 3) magn Cu í plöntum og sauðfé virðist vera nægjanlegt; 4) hæsta járnmagn, sem var í heysýnum frá riðubæjum, kann að vera við eitrunarmörk fyrir plöntur; 5) minnst var af Fe í heyi frá riðulausum bæjum, en hið gagnstæða gilti um Mn (og að nokkru um Zn); 6) mikið Mn í heyi kann að vera varnandi gegn uppkomu klínískrar riðu; 7) mikið Fe í heyi frá riðubæjum kann, vegna bælingar á Mn, að hvata uppkomu sjúkdómsins; 8) ítarlegar rannsóknir á Fe og Mn (helst samfara rannsóknum á Cu og Zn) í jarðvegi og heyi á sauðfjárbúum gætu bætt skilning á því hvers vegna riða kemur endurtekið upp á sumum bæjum en ekki á öðrum bæjum, jafnvel í næsta nágrenni við riðubæi.

INTRODUCTION

Inorganic elements found in Earth´s crust, soils, plants, animals or humans are subdivided into two categories: *trace elements*, also called *microelements*, found in small amounts, and *macroelement*s, often found in far greater amounts than are the microelements.

The general consensus is seemingly to confine trace elements to inorganic substances only: metals and metalloids. On the other hand it is more a matter of opinion where to set the so-called *trace limits*. In natural media, outside definite organisms (rocks, soils or elsewhere), the trace limits have been defined as less than 0.1% (less than 1 g kg^{-1}). In tissues of plants or animals the dividing line has been put one order of magnitude lower, i.e. less than 0.01% (less than 100 mg kg^{-1}) (Adriano 2001). These limits are not infallible as some elements that are generally defined as trace elements may at times occur in higher concentrations, especially in rocks or soils. Thus iron is often, strictly speaking, rather a macroelement than a microelement, and the same may also on occasion apply to manganese. Nevertheless, in biochemical terms, iron and manganese rather belong to trace elements than macroelements and are grouped accordingly.

Certain trace elements are essential to plants, animals or man in low *concentrations* or *amounts* that are required to support health, growth and reproduction when all other food ingredients are optimal. The *critical concentration* or *amount* may be defined to represent the lowest *sufficient concentration* or *amount* of any trace element that is essential for the health of a particular species (plants, animals, man). Prominent among these elements are: cobalt, copper, iron, manganese, molybdenum, selenium and zinc, which are dealt with in this presentation. Included with this group of trace elements are also chromium and iodine (Damgaard Poulsen 2005). These substances may, however, as Adriano (2001) points out, cause toxic symptoms if they are present or administered in excessive amounts.

A general review of data pertinent to the seven trace elements discussed here is given in Table 1.

Essential trace elements are often part of an active site in metalloenzymes (enzymes which are usually inactive without the metal elements), or may otherwise participate as cofactors (activators) in enzymic activity. They are in certain instances essential parts of vitamins and hormones as well, and may also participate in other vital functions (such as oxygen transfer). Insufficient amounts of these trace elements may result in deficiency symptoms which can be amended by administration or application of the particular trace elements. It should nevertheless be kept in mind that discolouring of plant leaves (chlorosis) or otherwise damaged foliage or undernourishment of animals due to deficiency of several trace elements can have many features in common. Thus deficiency symptoms are not necessarily specific in appearance, especially not in plants.

In our research on trace elements in forage and the occurrence of scrapie in sheep in Iceland, farms have been divided into four categories (Jóhannesson et al. 2004a). **Category 1:** Farms located in counties where scrapie has never been detected or located in areas never affected in otherwise afflicted counties. **Category 2:** Farms never afflicted by scrapie, or afflicted and restocked prior to 1960, but located amongst scrapie-prone or scrapie-afflicted farms in scrapie-afflicted counties (same localities as Categories 3 and 4). **Category 3:** Farms afflicted by scrapie after 1980 and afterwards restocked with

Table 1. A review of data pertinent to the seven trace elements discussed in the text (mostly from Adriano 2001).

Name	Atomic weight*	Specific gravity*	Occurrence in Earth's crust**	Deficiency symptoms Plants	Deficiency symptoms Animals	Deficiency symptoms Humans	Toxic symptoms Plants	Toxic symptoms Animals	Toxic symptoms Humans	Remarks
Manganese (Mn)	54.9	7.2	0.5-1.5 g kg^{-1}	Yes	Yes	?	Yes	?	Yes	Toxic to plants in heavily acidic soil. Demand differs after plant species. Little toxicity in animals.
Copper (Cu)	63.6	8.9	ca. 50 mg kg^{-1}	Yes	Yes	Yes	Yes	Yes	Yes	Toxic to plants but rather little to animals. Severe deficiency symptoms known in plants and animals.
Molybdenum (Mo)	95.9	10.2	1-2 mg kg^{-1}	Yes	Yes [1)]	-	Yes [1)]	Yes	-	Demand differs after plant species. Toxicity in animals mainly due to complexing of copper.
Selenium (Se)	78.9	4.8 / 4.3	50-90 µg kg^{-1}	-	Yes	Yes	Yes	Yes	Yes	Selenium is not considered essential to plants. Land areas with low Se are widespread in the world.
Cobalt (Co)	58.9	8.9	20-30 mg kg^{-1}	-	Yes	-	Yes	-	Yes	Cobalt is not considered essential to plants. Little toxicity in animals and humans.
Zinc (Zn)	65.4	7.1	ca. 70 mg kg^{-1}	Yes	Yes	Yes	Yes	?	Yes	Demand differs after plant species. Skin symptoms in Zn deficiency. Zn may interfere with Cu status.
Iron (Fe)	55.8	7.9	50-100 g kg^{-1}	Yes	Yes	Yes	Yes	Yes	Yes	Iron deficiency (indirect) first occurs in younger parts of plants. Ample Fe status in grass.

Symptoms: Yes: confirmed; ?: possible / probable, but not confirmed; - : uncertain / not known.
*: approximate values; **: usual average numbers.
[1)]: only demonstrated in experiments.

healthy sheep. **Category 4:** All farms (except one) where scrapie was diagnosed from April 2002 to March 2004. Collectively the farms in Categories 1 and 2 are referred to as *scrapie-free*. Farms in Category 3 are referred to as *scrapie-prone* and farms in Category 4 as *scrapie-afflicted*.

Forage samples, mainly round-bale silage, were collected from 47 farms in all categories at different locations from the summer harvests 2001-2003 and analysed for manganese, copper and iron (each metal in about 170 samples), molybdenum, cobalt and zinc (Mo and Co in about 115 samples, respectively, and Zn in 170 samples, from the 2003 harvest only) and selenium (about 90 samples from the harvests in 2002 and 2003) (Figure 1). Farms in Category 1 were not included in the selenium studies. As our main purpose was to study the possible relationship between the trace elements in the forage of sheep and the occurrence of clinical scrapie in Iceland the results have been discussed in terms of this ideology and published in several articles during the last few years: Jóhannesson et al. 2004a (copper, manganese), Jóhannesson et al. 2004b (selenium), Jóhannesson et al. 2004c (copper, manganese, selenium), Jóhannesson et al. 2005b,c (molybdenum) and Gudmundsdóttir et al. 2006a (iron, manganese). These papers, and our data on

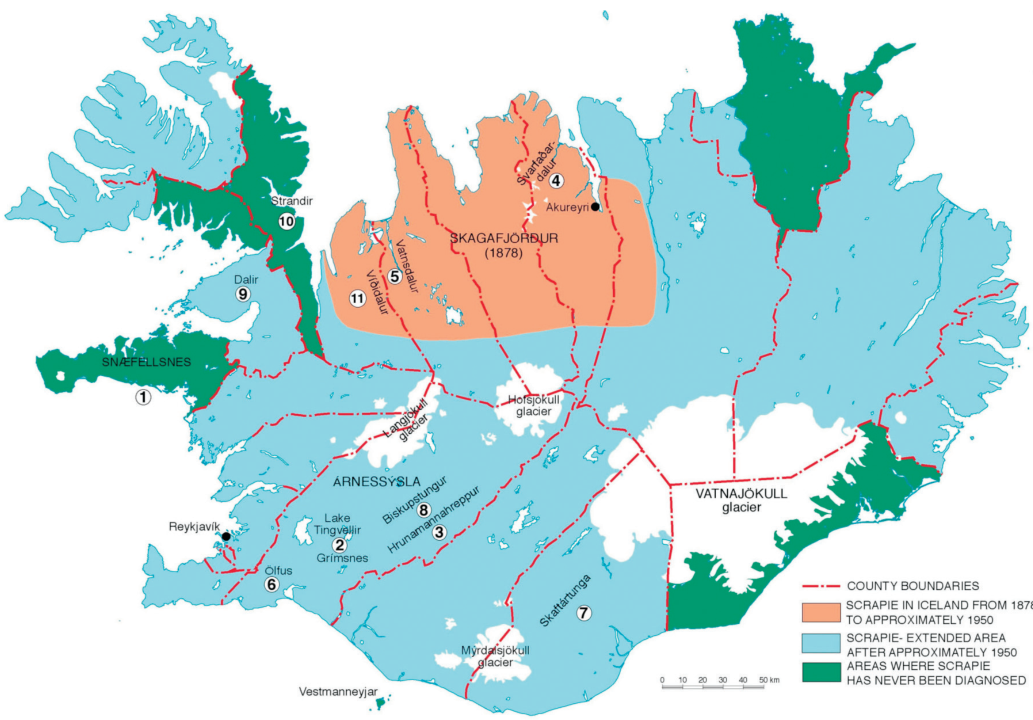

Figure 1. Scrapie in Iceland and locations of farms. From its presumed origin in Skagafjörður in 1878, scrapie was confined to a part of northern Iceland until ca. 1950 (orange). It has since spread patchily to greater or lesser parts of all counties (blue) except for four (green). The numbers 1-11 indicate the eleven different locations of the 47 farms in all scrapie categories where forage samples of the summer harvests 2001-2003 were collected for Mn, Cu and Fe analyses. Mo, Co and Zn were analysed in the forage samples of the summer harvest in 2003 only from 36-47 of the farms in all scrapie categories and in the same eleven locations. Selenium was analysed in the forage samples of the summer harvests in 2002 and 2003 from 18 of the farms in all categories (exclusive Category 1) and situated in locations 3, 4, 5, 6 and 11 only. The large green area in the north-east indicates one of the main areas in the country used to provide healthy lambs to restock formerly scrapie-afflicted farms.

cobalt and zinc that have not been published previously, are the axis around which the discussion below on the relation between trace elements and scrapie evolves. The spread of scrapie to larger parts of the country (Figure 1) supports the notion that scrapie is primarily, at least, an infectious disease although accessory factors like genetical susceptibility (Thorgeirsdottir et al. 1999) and the effect of trace elements, the topic of the present writing, can be determinant in the pathogenic process. The clinical features and history of scrapie have been reviewed by Brown and Bradley (1998).

In this overview our data, and the data of others, are however viewed and considered in a broader sense than we have accomplished in previous publications. Thus the results are first considered in relation to the critical amounts of the seven trace elements in plants and knowledge of deficiency symptoms or toxic symptoms due to these trace elements in plants in Iceland. Secondly, the value of the trace elements is evaluated in relation to animal health on Icelandic farms, in cattle and especially in sheep, with special reference to scrapie. Thirdly, due notice is given to deficiency and toxic symptoms in man. Finally, at the head of the section on each trace element comments are given (in smaller type) on the possible mechanism of action of the individual trace elements in living organisms as well as on their occurrence in rocks and soils.

In the following text each trace element is discussed separately followed by a final section on discussion and conclusions. Amounts or concentrations of trace elements in forage samples mentioned in the text refer to dry matter. It should also be noted that in this text the term critical amount or concentration is in general used with special reference to plants.

MANGANESE

Manganese is a metal and by definition belongs to the heavy metals (specific gravity greater than 7.0). Manganese is the twelfth most abundant element in Earth´s crust and never occurs as the native metal. The Mn concentration in Earth´s crust varies to a considerable degree (Adriano 2001). In Icelandic basalt the Mn concentration is in the range 1200-2000 mg kg^{-1}. In a study at Skriduklaustur in eastern Iceland the total amounts of Mn in the soil ranged from 1100-2200 mg kg^{-1} (Gudmundsson et al. 2005). In Icelandic soil the amount of easily soluble manganese was considered relatively high, but variable (152-1365 mg kg^{-1}), with the highest value in the soil of uncultivated bogs (Sigvaldason 1992). Manganese constitutes an active site in several enzymes but it is especially an activating factor (cofactor) for many holoenzymes. Thus in plants manganese is found in enzymes that are essential for the metabolism of nitrogen and for energy production. Mn also participates along with iron in the synthesis of chlorophyl (Adriano 2001). In animals Mn activates enzymes, among a multitude of other enzymes, that participate in the biosynthesis of certain proteoglycans that are essential for the development of bone and cartilage. These enzymes rank among the glycosyltransferases (Keen & Graham 1989).

Roots of plants absorb Mn from soil as divalent manganese ions (Mn^{2+}). The bioavailability of Mn is dependent on the acidity and the redox potential of the soil and is greater in acidic soil (low pH) than in alkaline soil (Adriano 2001). Icelandic soil is generally of medium acidity (pH 5-6) (Sigvaldason 1992). This, along with the high amounts of Mn in Icelandic rocks and soils and the poorly aerated soils often found in Iceland (Th. Gudmundsson, personal information 2006), contributes to a generally high bioavailability of manganese in plants in Iceland. It should, however, be taken into consideration that critical amounts may vary according to plant species and that grasses,for example, are less sensitive to low levels of manganese than oats or barley (Sigvaldason 1992, Knudsen 2005). In plants the critical concentration may as a general rule be assumed to be about 20 mg Mn kg^{-1}, 20-500 mg kg^{-1} may be considered as sufficient and a concentration in excess of 500 mg kg^{-1} may be toxic to the plants (Adriano 2001). In barley, however, toxic symptoms may occur with a lower Mn concentration or in the range of 100-300 mg kg^{-1} (Adriano 2001). A special reciprocal relationship seemingly exists between iron and manganese in plants (see iron below).

The lowest average concentration of manganese in Icelandic grasses (77 samples in six categories) was 51 mg kg^{-1} and the highest 259 mg kg^{-1} (Gudmundsson & Thorsteinsson 1980). In our study on forage samples collected from several areas in the country (a total of 172 samples, Figure 1) individual manganese concentrations were in the range of 40-550 mg kg^{-1} (Jóhannesson et al. 2004a). The average concentration in the samples varied according to scrapie status of the farms and was in the range of 125-190 mg kg^{-1}. In samples from scrapie-free farms (categories 1 and 2) Mn concentration was significantly higher than in samples from scrapie-prone and scrapie-afflicted farms (Figure 2). The manganese concentration was also significantly higher in forage from scrapie-free farms in scrapie-free areas (category 1) than in forage samples from farms in the other three categories (not shown in Figure 2). This finding, especially, may indicate that high concentrations of manganese in the forage can have a preventive effect on the occurrence of scrapie in Iceland. As no statistical difference has been found between the manganese concentration in the blood of sheep on scrapie-free, scrapie-prone and scrapie-afflicted farms the idea was fostered that the protective effect of manganese might be confined to the cellular border of the gastrointestinal tract (Jóhannesson et al. 2005a) (See also Discussion and Conclusions).

Under *in vitro* conditions substitution of manganese in place of copper in the normal prion protein (PrP^{c}) results in conversion of the protein to an abnormal isoform reminiscent of the pathological prion protein (PrP^{sc}). Conversion of the protein by manganese has not been shown to occur *in vivo* and the *in vitro* fabricated protein has not been shown to be infectious (Brown 2002). Thus the effect of manganese in these *in vitro* experiments is not easy to interpret.

Results of the two studies referred to above indicate that Mn concentration in grasses in Iceland varies and is in general high. The Mn concentration in oat leaves in Iceland may, however, be insufficient at times, especially if the soil is dry and with a high pH (Sigvaldason 1992).

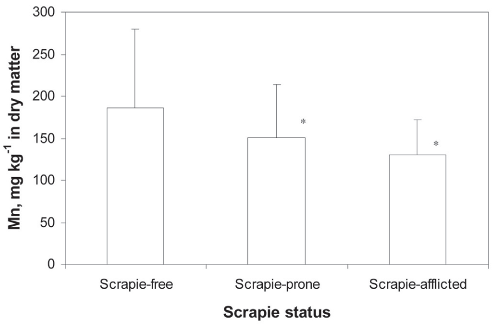

Figure 2. Mean concentration of manganese in forage samples from 47 scrapie-free, scrapie-prone and scrapie-afflicted farms. Vertical bars show the standard deviation (from Jóhannesson et al. 2004a).
* significantly lower concentration than in samples from scrapie-free farms ($P < 0.05$, ANOVA).

We are not aware of any clinical manifestations of Mn deficiency in sheep or cattle in Iceland at the present time. Mn deficiency may result in decreased or deranged synthesis of bone and cartilage, especially in birds (perosis). Symptoms of that kind do not seem to occur in Iceland (Jarle Reiersen, personal information 2005). There are few if any reliable data on manganese toxicity in animals (Table 1).

Acute manganese intoxication (pneumonitis) is known in humans due to inhalation of manganese dust. Chronic manganese intoxication in humans is known to result in neurological symptoms of the Parkinsonian type (Goyer 1996, Goldhaber 2003).

COPPER

Copper belongs to the heavy metals and it is ranked 26th among the elements in crustal abundance. In nature Cu forms many compounds with sulphur and other components and it also occurs as native metal. The amount of copper found in rocks is often about 50 mg kg^{-1} (Adriano 2001, Table 1). In basalt the copper content may be as high as 90 mg kg^{-1}. In the study at Skriduklaustur in eastern Iceland the total amount of copper in soil was on average 50 mg kg^{-1} (Gudmundsson et al. 2005). In Icelandic soil the amount of easily soluble copper is relatively high (7-13 mg kg^{-1}) (Sigvaldason 1992). Copper enzymes participate in energy production in both plants, animals and man. In plants Cu enzymes are essential for the synthesis of lignin in cell walls and copper deficiency is at first manifested as decreased growth of seeds and fruits (Adriano 2001). In animals many Cu enzymes are essential to life. One of the most conspicuous Cu enzymes is essential for the synthesis of connective tissue. Thus in case of Cu deficiency rupture or decreased synthesis of connective tissue may be seen almost anywhere in the body (central nervous system, bones, vessels, etc.). Other Cu enzymes are essential for the synthesis of keratin in hair, claws or hooves and for the permanence of hair colour, and Cu is a part of the normal prion protein (PrP^c). Cu is also an active site in some so-called antioxidative enzymes that regulate oxygen metabolism in the body (Keen & Graham 1989, Pena et al. 1999, Goyer 1996, Frank 1998, Jóhannesson et al. 2003).

Roots of plants absorb copper from soil as divalent Cu ions (Cu^{2+}) and probably also bound to organic substances and in the form of inorganic complexes. The bioavailability is in general lesser than is the case for other trace elements. The bioavailability is greatest in acidic soil (pH lower than 5-6). In plants the critical amount may generally be assumed to be about 5 mg Cu kg^{-1}, 5-20 mg kg^{-1} may be considered as sufficient, and concentrations in excess of 20 mg kg^{-1} may be toxic to plants (Adriano 2001).

The lowest average concentration of copper in Icelandic grasses (77 samples in six categories) was 3.70 mg kg^{-1} and the highest 4.65 mg kg^{-1} (Gudmundsson & Thorsteinsson 1980). In our study (Jóhannesson et al. 2004a) on forage samples collected from several areas in the country (a total of 172 samples, Figure 1) individual copper concentrations ranged from 4-16 mg kg^{-1}. The average concentration was 8-9 mg kg^{-1} in samples from farms in all scrapie categories and did not differ significantly between groups. The Mn/Cu ratio was found to be significantly higher in samples from scrapie-free farms than in samples from scrapie-afflicted farms but not in samples from scrapie-prone farms (Figure 3). The Mn/Cu ratio was however significantly higher in samples from scrapie-free farms in scrapie-free areas than in samples from farms in the other three categories (not shown in Figure 3).

Previous research by Pauly & Harris (1998) and Sigurdsson et al. (2003) have shown that copper may facilitate the endocytosis of the prion protein and administration of a copper chelator significantly delayed the onset of prion disease in experiments with mice. Copper may also promote the conformational changes in the normal prion protein (PrP^c) leading to the pathological isoform (PrP^{sc}) (McKenzie et al. 1998). On the basis of the results of these authors Jóhannesson et al. (2004a) therefore forwarded the idea that high concentrations of copper in forage, relative to manganese, would favour the occurrence of clinical scrapie.

The results of the former study quoted above (Gudmundsson & Thorsteinsson 1980) might indicate that copper deficiency had existed in grasses at that time in certain areas in the country. Neither the results of the second and more recent study (Jóhannesson et al. 2004a), nor the Icelandic data from the Swedish-Icelandic comparative study of Kirchmann et al. (2005) support that notion. In addition, to our knowledge there are at the present time no known cases of copper deficiency in plants in Iceland.

There are at the present time no available data on copper deficiency in sheep or cattle in Iceland. Symptoms of possible copper deficiency might have occurred previously in cattle that conspicuously lost their normal hair colour in the head, especially around the eyes, and became greyish in appearance (see

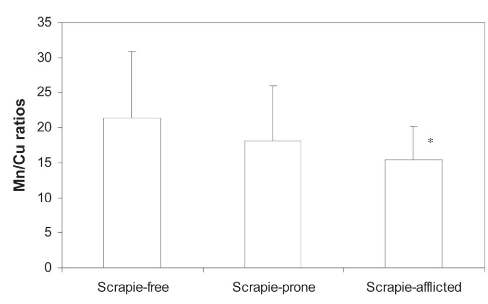

Figure 3. The calculated mean manganese/copper ratios in forage samples from 47 scrapie-free, scrapie-prone and scrapie-afflicted farms. Vertical bars show the standard deviation (from Jóhannesson et al. 2004a). * significantly lower ratio than in samples from scrapie-free farms (P< 0.05, ANOVA).

Cu enzymes above). Staggering gait in young lambs (called in Icelandic *fjöruskjögur*) previously occurred on farms where the sheep grazed on seaweeds and also occasionally on farms not located near the coast. Pálsson & Grímsson (1953, 1954) found a severe degree of demyelination (defect in connective tissue in neuronal sheaths) in the brain of these lambs and demonstrated that the disorder could be largely prevented by giving a supplement of copper to the pregnant ewes. Copper intake was normal in the ewes feeding on the seaweed although the copper concentration in the blood was low. In the light of later research the most logical explanation for this disease might have been high levels of molybdenum or zinc in the seaweed, as both metals may interfere with the bioavailability of copper (see below). In this context it is of considerable importance that Scottish scientists (Maclachlan & Johnson 1982) have found low levels of copper and molybdenum in seaweed and at the same time high concentrations of zinc and sulphur.

Recent studies moreover show that the concentration of copper in the blood of ewes in Iceland is within normal limits. The same might apply to the activity of SOD1, a copper-zinc enzyme, in erythrocytes (Jóhannesson et al. 2005a). It was therefore concluded that at the present time the copper status of Icelandic sheep is sufficient. It should, however, be kept in mind that sheep are more sensitive to variation in copper status than most other animals (Søli 1980, Lockhart & Mercer 1999).

Copper deficiency and copper intoxication are seldom seen in humans. Two or three genetically determined diseases have been described in man, where either copper accumulates resulting in tissue damage or is missing in vital enzymes. The best known of these is Wilson´s disease (Goyer 1996). Zinc is used for treatment of Wilson´s disease as it induces intestinal cell metallothionein which blocks copper absorption and also reabsorption of endogenously secreted copper (Brewer et al. 1998). Tetrathiomolybdate is also used for treatment of Wilson´s disease (see molybdenum).

MOLYBDENUM

Molybdenum belongs to the heavy metals and ranks 53rd in crustal abundance. The concentration in rock is often in the range of 1-2 mg kg^{-1} but much higher concentrations are found (Adriano 2001, Table 1). In Icelandic basalt the molybdenum concentration is generally 1 mg kg^{-1} and in the soil of uncultivated fields in eastern Iceland the total amount is in the range of 0.5-0.8 mg kg^{-1} (Sverrisson & Dalmannsdóttir 2005). Molybdenum is (along with iron) an active site in two essential enzymes that contribute to the fixation of nitrogen from the air and nitrate from the soil and transformation of nitrogen into ammonia in plants. Ammonia is the first step in the synthesis of amino acids, the building blocks of proteins. Because of these functions molybdenum is ubiquitously found in vegetables. The primary symptom of molybdenum deficiency in plants is accordingly reminiscent of stunted growth due to an insufficient supply of nitrogen. Molybdenum is also found in several essential enzymes in animals and humans (Goyer 1996, Adriano 2001).

Roots of plants absorb molybdenum primarily as molybdate (MoO_4^{2-}) and Mo bioavailability is highest in soils of low acidity or in alkaline soil. The concentrations of Mo in plants varies to a great extent, as does the critical concentration. In most plants the critical concentration is in the range of 0.1-1.0 mg kg^{-1}. In contrast to most essential trace elements there are in general no upper limits of Mo concentration that can be associated with toxic symptoms in plants. Thus the toxic effect of Mo in plants has only been demonstrated experimentally (Adriano 2001, Table 1). Clover may require relatively high amounts of molybdenum for normal growth (Sverrisson & Dalmannsdóttir 2005). It is also known that the Mo amounts available may be insufficient for cultivation of cauliflower in Iceland (S. Ólafsson, personal information 2005). Furthermore in the somewhat older literature there are indications that symptoms of molybdenum deficiency can occur in rape in Iceland (Guðleifsson & Gunnarsson 1978).

Molybdenum was determined in 115 forage samples from various localities of the summer harvest in 2003 (Figure 1). The mean molybdenum concentration was 0.23 mg kg^{-1}, with a wide range of individual results from

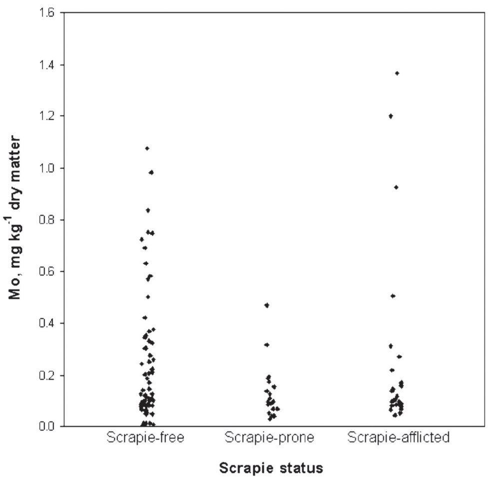

Figure 4. Distribution of individual molybdenum concentrations in forage samples from 36 scrapie-free, scrapie-prone and scrapie-afflicted farms (from Jóhannesson et al. 2005b).

0.0043 mg kg^{-1} to 2.37 mg kg^{-1}. There was no significant difference between the mean Mo concentration in forage samples from farms in the various scrapie categories (Jóhannesson et al. 2005b,c). The collective distribution of individual results is shown in Figure 4. From the above data it was concluded that the molybdenum concentration in forage of sheep was often in the low normal range or perhaps lower.

In the presence of ample sulphide as in the rumen of sheep and other ruminants molybdenum may form tetrathiomolybdate (or other thiomolybdates) that actively chelate and inactivate copper or may even be absorbed and interfere with copper availability inside the body. If molybdenum concentration in the forage is high, and especially if the ratio between copper and molybdenum (the Cu/Mo ratio) is lower than 4, this may lead to copper deficiency in the animals (Ward 1978, Frøslie et al. 1983, Adriano 2001, Spears 2003). In our studies the Cu/Mo ratio was always higher than 4 (the lowest individual Cu/Mo observed ratio was 6) (Jóhannesson et al. 2005b,c). These results are in support of the contention that there are no obvious signs of copper deficiency in sheep in Iceland at the present time (Jóhannesson et al. 2005a).

Molybdenum intoxication is known abroad in sheep and cattle on pastures with high molybdenum content. Copper sulphate supplement in the diet prevents the toxicity (Goyer 1996). Frank (1998) has pointed out the strong similarities between copper deficiency and molybdenosis in cattle and sheep. Molybdenum has little or uncertain toxicity in humans. Deficiency symptoms are probably not known in humans and have only been demonstrated experimentally in animals (Table 1). The low degree of toxicity has helped promote an increase in the use of molybdenum in the metal industry (high-temperature resistant steel alloys, etc.).

Determination of sulphur was included in the molybdenum study. Sulphur concentration was remarkably constant (mean concentration of sulphur was 2.34 mg kg^{-1} in samples from scrapie-free farms, 2.36 mg kg^{-1} in samples from scrapie-prone farms and 2.29 mg kg^{-1} in samples from scrapie-afflicted farms).

SELENIUM

Selenium is different from the other trace elements discussed in this text as it is primarily classified as a metalloid although it has properties of both metals and nonmetals. Selenium is widespread in rocks, most often in small amounts (it is ranked 68th in crustal abundance), and often as selenide associated with heavy metal sulphides formed during volcanic eruptions. The selenium concentration in the Earth's crust is often in the range of 50-90 µg kg^{-1} and the total amount in soil is in general in the range of 100-2000 µg kg^{-1}. Soils having a concentration higher than 2000 µg kg^{-1} are likely to be seleniferous and may, especially in arid or semiarid areas, induce toxic symptoms in plants. Areas with low selenium concentration in the soil occur much more widely in the world than areas with high concentration. It is, however, a well known fact that total selenium in soils is not a reliable index of plant available Se (Adriano 2001). Selenium concentration in soils in Iceland is in general considered to be high. Thus almost 3000 µg kg^{-1} Se was found in a soil at the Institute for Experimental Pathology at Keldur in Reykjavík (Símonarson et al. 1984). Although selenium is not considered essential to plants it may, as mentioned, in high amounts cause toxic symptoms in plants (Table 1). In mammals there are several so-called selenium proteins where Se is bound to amino acids like sulphur. As far as is known the enzyme glutathione peroxidase (GPX) is by far the most important of these proteins and the essentiality of selenium for man and other mammals relates to the activity of the GPX isoenzymes. There are at least four known GPX isoenzymes. Best known is the GPX enzyme in erythrocytes and plasma and the activity of this isoenzyme is often positively correlated with the Se concentration in the blood of domestic animals. Determination of GPX activity in the blood of sheep or other domestic animals (cattle, horses) has accordingly been taken as an index of Se concentration in the blood (Eiríksdóttir et al. 1981, Símonarson et al. 1984, Símonarson 1986, Jóhannesson et al. 2004b). Glutathione peroxidase is an important antioxidative enzyme and is functionally associated with the Cu-Zn enzyme SOD1 (Jóhannesson et al. 2003). Deficient GPX activity due to insufficient intake of Se, or for other reasons, may result in oxidative tissue damage in many organs (e.g. skeletal muscles, heart and diaphragm) or in functional irregularities in various organs (e.g. the maintenance of the corpus luteum and other functions associated with pregnancy). Tocopherol (vitamin E) has a synergistic action with GPX and may to a certain extent ameliorate Se deficiency in animals (Símonarson 1986, Adriano 2001, Jóhannesson et al. 2004b). In this context due notice should be given to the fact that Zn may be an activating factor (coenzyme) for GPX activity (see zinc below).

Selenium is absorbed in roots of plants in the form of selenate, selenite or organic Se compounds. In alkaline soil most of the Se is absorbed as selenate and the bioavailability is relatively high. If the soil pH is neutral or acidic Se is preferably accessible as selenite and the bioavailability is much lower (Adriano 2001). As the soil is rather acidic in Iceland, often poorly aerated and abundant in iron and aluminium oxides that bind selenites, it is to be expected that the selenium concentration in Icelandic plants is low in spite of the high total amounts found in soil (Th. Gudmundsson, personal information 2006).

At the experimental farm Hvanneyri in western Iceland Se was determined in hay and silage from November 1979 to May 1980. Selenium concentration in the samples ranged from 38-144 μg kg^{-1} (Eiríksdóttir et al. 1981). The same group of authors determined Se in 136 hay samples from the northern, southern and southwestern parts of the country and found Se in an average concentration of 40, 67 and 72 μg kg^{-1}, respectively (Símonarson et al. 1984). On the other hand in a few samples of vegetation from the highlands and seaweeds the average Se concentration was 207 μg kg^{-1} and 218 μg kg^{-1}, respectively (Símonarson et al. 1984). In our study (Jóhannesson et al. 2004b) selenium was determined in 88 forage samples collected on scrapie-free, scrapie-prone and scrapie-afflicted farms (Figure 1). The average Se concentration was 21.1 μg kg^{-1}. The mean Se concentration did not differ significantly between the three categories. In the sample collection there were in all categories a few samples with Se concentration in the 50-100 μg kg^{-1} range. These samples were all collected late in the summer (mown late) and most of them happened to be from scrapie-afflicted farms. Purdey (2000) has previously found Se concentration in unspecified grazing plants (herbage) in two farming areas in northern Iceland similar to what we found in the forage samples.

According to foreign data symptoms of Se deficiency may be seen in animal husbandry if the Se concentration in the feed is appreciably lower than 100 μg kg^{-1}. It is therefore no wonder that veterinarians see symptoms of Se deficiency in lambs ubiquitously in the country, and also to some extent in heifers and foals, in spite of widely practiced prophylactic injections of selenite and vitamin E or the use of mineral mixtures containing selenium (Jóhannesson et al. 2004b).

In the blood of ewes the Se concentration varies from autumn to the following spring. In autumn, shortly after the ewes have been gathered from the highlands, the concentration is considered sufficient (above 100 ng ml^{-1}) but in the spring, when the ewes are pregnant and have been housed for months and fed inside the sheds, the concentration is near deficiency levels (50-100 ng ml^{-1}). This is in accordance with the fact that the Se concentration is higher in plants in the highlands than in forage from cultivated plots (cf. above). Additionally, pregnancy may be a tax on the Se in mothering ewes (Eiríksdóttir et al. 1981, Jóhannesson et al. 2004b).

The most prevalent symptom of Se deficiency is known as white muscle disease, an affliction of skeletal muscles most often seen in newborn or young lambs. The lambs have difficulties in standing on their legs, especially the hind legs, and display a shaky and feeble gait in the pen. This disease is very pointedly called *stíuskjögur* in Icelandic (stía = pen, *skjögur* = shaky gait). It has been amply substantiated from the verbal rendering of older farmers and veterinarians that white muscle disease in lambs was not known in Iceland before 1960. Around that time it became customary for the majority of farmers to feed the sheep inside the sheds during the winter instead of having the sheep grazing outside during winter time. Apart from white muscle disease, selenium deficiency may result in cases of fatal diaphragm rupture in sheep.

Se deficiency has been associated with retained placenta in cows (Þorkelsson 1997) and mastitis in heifers (Arnþórsdóttir 2002). In both of these studies GPX activity in blood was used as an index of Se concentration in blood. The same applies to a study of Stefánsdóttir and Árnadóttir (2005) in horses. In the study of Arnþórsdóttir (2002) it is of considerable interest that the GPX activity in the blood of all the heifers included, whether affected with mastitis or not, was considered low. In our opinion stringent criteria should be used if GPX activity in blood is to be used as an index of Se concentration. This is not least due to the fact that the correlation between GPX activity and Se concentration varies and is low in pregnancy (Jóhannesson et al. 2004b). Perhaps GPX activity in the blood may be a better index of Se concentration in the blood of male than female animals. One study of that kind has recently been performed in rams (Sigurðarson et al. 2006).

Selenium toxicity in plants has been described in some seleniferous areas where the soil is rich in selenium, contributing to high selenium in the vegetation (Goyer 1996). There are no known cases og Se toxicity in animals in Iceland. In China selenium toxicity in man has been observed in seleniferous areas. The most extensively documented deficiency of selenium in humans has also been described in China, a cardiomyopathy called Keshan disease that is most often seen in children younger than 15 years (Goyer 1996). There are no indications of selenium deficiency in humans in Iceland (Jóhannesson et al. 1981, Thorgeirsdóttir et al. 2005).

COBALT

Cobalt belongs to the heavy metals. Values for crustal abundance of Co usually range from 20 mg kg^{-1} to 30 mg kg^{-1}, ranking Co 30th among the elements. Thus cobalt is less abundant in Earth´s crust than copper or zinc (Table 1). Total amounts of cobalt in soil may vary to a great extent or from 2 to 40 mg kg^{-1} (Adriano 2001). In a study at Skriðuklaustur in eastern Iceland the total concentration of cobalt in soil varied considerably but was on average 18 mg kg^{-1} (Gudmundsson et al. 2005). Cobalt has not been substantiated as an essential factor of plants (or at least not directly). Cobalt is a constituent of vitamin B12 (medical preparations of vitamin B12 contain either cyanocobalamin or hydroxocobalamin) which is required by all animals for, among other functions, the production of red blood cells. In the animal organism vitamin B12 is transformed into two active coenzymes, methylcobalamin and 5-deoxyadenosylcobalamin. Methylcobalamin is required for the formation of methionine from homocysteine. The biological value of Co is in general related to the activity of these coenzymes (Goyer 1996, Adriano 2001, Hillman 2001). In nature, the primary sources of vitamin B12 or like substances are certain microorganisms that grow in soil, sewage, water or the intestinal lumen that synthesize the vitamin (Hillman 2001). Low levels of Co in feedstuff can cause deficiency symptoms in cattle or sheep due to insufficient production of the vitamin (Goyer 1996, Adriano 2001). Ruminants, especially sheep, are considered to have greater demand for vitamin B12 than other animal species. Microorganisms in the rumen produce the vitamin (or its congeners) which the animals may subsequently utilize to some extent (Ulvund 1995, Whitehead 2000).

Several factors may influence the absorption of Co in the roots of plants. Thus a high content of iron and manganese oxides in soil impairs the availability of Co to plants. The bioavailability of Co is greater in acidic soil than under alkaline conditions. The dissociable form of Co available for absorption in roots of plants is most likely primarily the divalent cation, Co^{2+}. The concentration of Co in plants varies. The Co concentration is relatively low in grasses compared to other plant species. The general consensus is that the critical concentration of Co in the feed of sheep and cattle should not be lower than approximately 70-100 µg kg^{-1} (Whitehead 2000, Adriano 2001).

The lowest average concentration of cobalt in Icelandic grass (77 samples in six categories) was 120 µg kg^{-1} and the highest 420 µg kg^{-1} (Gudmundsson & Thorsteinsson 1980). In our own study (results not previously published) cobalt was determined in 112 samples of the summer harvest in 2003 from various localities (Figure 1). The mean Co concentration was 317 µg kg^{-1} (range 41 - 2010 µg kg^{-1}). In this study fourteen individual values ca. 12% were below 100 µg kg^{-1} and three were very high (above 1000 µg kg^{-1}). The mean concentration did not differ significantly in samples from farms in the four scrapie categories (range of means 262 - 357 µg kg^{-1}). In conclusion the results of our study indicate that Co deficiency might occur occasionally in sheep in Iceland.

Cobalt deficiency in sheep occurs in many countries and it is possibly often undiagnosed. It is most often seen in grazing animals, especially in growing lambs. The symptoms vary and are generally unspecific. In its mild form Co deficiency may result in stunted growth, loss of appetite and weight loss – and more specifically in running tears and changes in wool texture. Co deficiency in sheep is associated with low vitamin B12 activity in plasma and higher than normal levels of homocysteine in the serum of the animals (Ulvund 1995).

In high concentrations Co may induce chlorosis and necrosis in plants (Adriano 2001). Co toxicity in animals is either uncertain or unknown and it is low in humans. Deficiency symptoms in man due to insufficient intake of Co are most likely not known. Thus pernicious anemia and other vitamin B12 related deficiency symptoms in man are virtually never caused by insufficient availability of Co in the food but are most often caused by deficient production of the so-called intrinsic factor, resulting in lack of absorption of the formed vitamin (Hillman 2001).

ZINC

Zinc belongs to the heavy metals and is the 24th most abundant element in the Earth´s crust, with an average value of ca. 70 mg kg^{-1}. Zinc is thus in general somewhat more abundant than copper (Table 1). Most of the Zn used comes from ores containing Zn sulphide minerals (Adriano 2001). In a study at Skriðuklaustur in eastern Iceland the total Zn concentration in soil was on average 56 mg kg^{-1} (Gudmundsson et al. 2005). In another study the amount of easily soluble Zn in Icelandic soil was generally in the range of 10-17 mg kg^{-1} (Sigvaldason 1992). Zinc is an essential element for the growth and development of plants, animals and microorganisms and is ubiquitous in the environment. Zinc has a high affinity for nitrogen and sulphur ligands in proteins or amino acids, which may account for many of its biological effects. Zinc has a general stabilizing effect on cell membranes by complexing thiol groups as well as phospholipids in the membranes. It also stabilizes the stucture of DNA and RNA and ribosomes, and nucleic acid polymerase activity is dependent on zinc. Zinc chelates with cysteine and/or histidine are found in so-called "zinc fingers" that are essential for the activity of some transcription factors. Last, but not least, Zn functions with more than 200 enzyme systems, often as tightly bound cofactor of metalloenzymes, activating enzymic activity. Thus Zn is a cofactor of collagenase, a major structural factor in the extracellular matrix. Thus, wound healing and the integrity of cell membranes are highly dependent on zinc and may explain why symptoms of Zn deficiency in man and animals are often first seen in the skin and as delayed wound healing (Scott & Eagleson 1988, Goyer 1996, Chan et al. 1998, Adriano 2001). Zn may be an activator of GPX activity, either directly as cofactor, or indirectly through the activity of superoxide dismutase (SOD1), a Cu-Zn enzyme (Virgilli et al. 1999, Xiang et al. 2004). This interaction might lead to erroneous results if GPX activity in the blood is taken without scrutiny as an index of the Se concentration in the blood (see selenium above).

Zn is absorbed in the roots of plants primarily as the divalent cation (Zn^{2+}). The bioavailability is highly dependent on the acidity of the soil. In acidic soil the bioavailability is greater than under alkaline conditions. Thus Zn deficiency in plants most often occurs when the soil is alkaline. Nevertheless, Zn deficiency is well known also in plants growing in acidic soil, presumably due to concurrent absorption of high amounts of ferrous ions (see iron) (Adriano 2001). The critical concentration of zinc in plants varies according to plant species but it is generally considered to be about 20 mg kg^{-1}; concentrations in the range of 20-150 mg kg^{-1} are considered as sufficient and concentrations in excess of 100-150 mg kg^{-1} may cause toxic symptoms in the form of yield reduction and phytotoxicity similar to Fe chlorosis. In Zn deficiency it is noteworthy that the permeability for phosphate is increased in the roots of plants. This increase may border on toxic effect in the plants (Adriano 2001).

The lowest average concentration of Zn in Icelandic grass (77 samples in six categories) was 22 mg kg^{-1} and the highest 39 mg kg^{-1} (Gudmundsson & Thorsteinsson 1980). In our study (results not previously published) on forage samples from several areas in the country (a total of 170 samples of the summer harvest in 2003, Figure 1) the Zn concentration was on average 35.3 mg kg^{-1} (range 14.1 - 85.0 mg kg^{-1}). The Zn concentration was in excess of 50 mg kg^{-1} in only 12 (7%) of the samples.

The mean Zn concentration was significantly higher ($P = 0.005$) in samples from scrapie-free farms in scrapie-free areas (41.4 mg kg^{-1}; $n = 28$) than in samples from scrapie-free farms in scrapie-afflicted areas (34.8 mg kg^{-1}; $n = 69$), samples from scrapie-prone farms (32.0 mg kg^{-1}; $n = 37$) and in samples from scrapie-afflicted farms (34.9 mg kg^{-1}; $n = 36$). The mean Zn concentration in samples from scrapie-free farms as a group (categories 1 and 2, n= 97) did not differ significantly from farms in the two other scrapie classes.

The results presented above indicate first that the Zn concentration in forage of sheep can be lower than optimal and thus on occasion result in Zn deficiency in the animals, and secondly that the Zn concentration in samples from farms in scrapie-free farms in scrapie-free areas was significantly higher than in samples from scrapie-free farms in scrapie-afflicted areas, scrapie-prone and scrapie-afflicted farms.

Zinc deficiency can result in parakeratosis in swine and other animal species, including grazing animals (Damgaard Poulsen 2005). The authors have on a few occasions observed cases of similar dermatological symptoms in both cattle and sheep. This is in accordance with the above conclusion that the levels of zinc in the forage of sheep or cattle may occasionally result in symptoms of zinc deficiency in the animals. Zinc toxicity in animals must be very low as experimental animals have been given up to 100 times their dietary requirements without any toxic effect (Goyer 1996).

In humans zinc deficiency may occur especially along with dietary inadequacies associated with chronic diseases like chronic alcoholism, malabsorption syndromes, ulcerative colitis, etc. After inhalation of zinc fumes a fever attack may develop 4-8 hours after exposure, simulating an acute malaria attack (Goyer 1996, Chan et al. 1998).

Acrodermatitis enterohepatica is a rare autosomal recessive disorder in man, due to impaired intestinal absorption and transport of zinc, that requires life-long oral zinc therapy (Chan et al. 1998). Zinc is a potent inducer of metallothionein synthesis in gastrointestinal mucosal cells and may be used in Wilson´s disease in order to decrease the absorption of copper (see copper).

Zinc has an adstringent effect and a weak antiseptic effect. Zinc compounds, especially zinc oxide, have traditionally been used for generations as dermatological remedies.

IRON

Iron is the second most abundant metal in the Earth´s crust after aluminium. Thus iron occurs ubiquitously in rocks and soils. In nature Fe forms compounds with oxygen and sulphur or other components and also occurs as the native metal. In basalt the iron concentration usually ranges from 70-110 g kg^{-1} but is less in rhyolite. In the study at

Skriðuklaustur in eastern Iceland total amounts of iron in the soil ranged from 94-111 g kg^{-1} (Gudmundsson et al. 2005). The amount of easily soluble Fe in Icelandic soil is considered to be relatively high and is in the range of 1-3.4 g kg^{-1} with the highest values in swamp areas, both in drained and cultivated peat and in uncultivated peat (Sigvaldason 1992). The amount of soluble iron is always low in comparison to the total amount of iron in the soil. In soil the solubility of Fe is dependent on the ratio between divalent (ferrous; Fe^{2+}) and trivalent iron ions (ferri; Fe^{3+}). In poorly aerated waterlogged soil, as often is found in Iceland, relatively high amounts of Fe exist as the soluble Fe^{2+} ion. Under these conditions reduction of the Fe^{3+} ion to the Fe^{2+} ion is brought about by anaerobic bacteria (Mengel & Kirkby 1987). On the other hand in well aerated soil, Fe^{3+} ions dominate over Fe^{2+} ions and react easily with hydroxyl ions, resulting in the formation of insoluble iron hydroxides. This may well contribute to Fe deficiency in plants growing on these soils. Plants may, however, circumvent this by forming ferri-complexes that are taken up in the roots, where trivalent iron is subsequently reduced and released as ferrous iron. The Fe^{2+} ion seems to be the main or the only absorbable form of iron in the roots of plants (Mengel & Kirkby 1987).

Iron is found in all living cells, often co-ordinately bound in the heme nucleus in hemoglobin or heme enzymes like the cytochromes. Cytochromes are found in all organisms and act as redox catalysts in respiration, energy conservation and photosynthesis. Catalase and peroxidases are other examples of iron heme enzymes. Iron also occurs in enzymes as functional sites without the heme nucleus (Scott & Eagleson 1988). Iron, along with manganese, is necessary for the formation of chlorophyll. In iron deficiency, like manganese deficiency, there is a lack of chlorophyll resulting in chlorotic leaves. However, Fe deficiency begins with paling of the youngest leaves whereas Mn deficiency often starts with the appearance of chlorotic spots on the older leaves (Mengel & Kirkby 1987). In humans and animals the principal symptom of iron deficiency is microcytic anaemia due to the lack of fully formed hemoglobin (Hillman 2001). The trivalent manganese ion (Mn^{3+}) and the trivalent iron ion (Fe^{3+}) are thought to be chemically similar. This might, perhaps, explain to some extent the special reciprocal relationship that seemingly exists between iron and manganese in plants (Mengel & Kirkby 1987, Adriano 2001; see also below).

In Icelandic soil iron seems to be soluble to such a great extent that iron deficiency is not likely to occur in grass species. Instead iron toxicity might occur, especially in plants growing in swamp areas (Th. Gudmundsson, personal communication 2006) and possibly also in grass growing on home fields. Data on the critical concentration of iron are not well documented as iron deficiency in plants may often be relative to disturbed distribution of iron within the plant organism (Mengel & Kirkby 1987).

The lowest average concentration of iron in Icelandic grass (77 samples in six categories) was 97 mg kg^{-1} and the highest 864 mg kg^{-1} (Gudmundsson & Thorsteinsson 1980). In our own study (Gudmundsdóttir et al. 2006a) on forage samples from various areas in the country (170 samples, Figure1) the iron concentration ranged from 57 mg kg^{-1} to 1379 mg kg^{-1}. In 14 of the samples (ca 8%) the iron concentration was less than 100 mg kg^{-1} and in three samples (ca. 1.8%) it was above 1000 mg kg^{-1}. It was considered that the three samples with iron concentration above 1000 mg kg^{-1} were contaminated by soil, and these samples were thus excluded from further processing. The mean iron concentration was significantly higher in forage from the scrapie-afflicted farms than in forage from farms in the other scrapie categories (Figure 5). The mean iron concentration was in fact lowest in forage samples from the scrapie-free farms in scrapie-free areas although it did not differ significantly from the mean iron concentration in forage samples from scrapie-free farms in scrapie-afflicted areas (Gudmundsdóttir et al. 2006a).

In plants iron and manganese are seemingly biochemical antagonists resulting in a reciprocal relationship between the concentrations of these two elements. Although the mechanism behind this antagonism is not fully known it may involve concurrence between the two metals for absorption in the roots of plants as well as concurrence for binding to enzymes (Mengel & Kirkby 1987, Adriano 2001). By using the results from the manganese determination in the same forage samples (Jóhannesson et al. 2004a), the Fe/Mn ratios were calculated for samples from farms in dif-

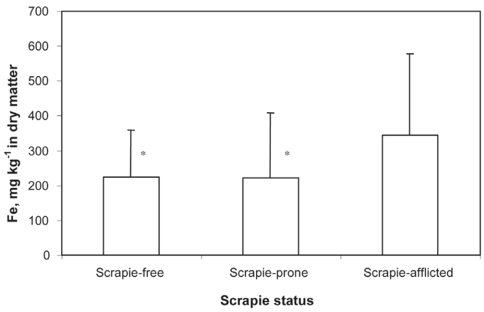

Figure 5. Mean concentration of iron in forage samples from 47 scrapie-free, scrapie-prone and scrapie-afflicted farms. Vertical bars show the standard deviation (from Gudmundsdóttir et al. 2006). * significantly lower concentration than in samples from scrapie-afflicted farms (P<0.05, ANOVA).

ferent scrapie categories. The results are shown in Figure 6. According to Adriano (2001) the Fe/Mn ratio should be in the range of 1.5-2.5 in healthy plants. If, on the other hand, the ratio exceeds 2.5, it suggests a relative overactivity of iron compared to manganese, and if it is less than 1.5 it suggests a relative dominance or overactivity of manganese over iron. The Fe/Mn ratios in the present study (Gudmundsdóttir et al. 2006) suggest that in the forage from scrapie-afflicted farms iron may be predominant over manganese, in forage from scrapie-prone and scrapie-free farms the metals are in balance (Figure 6), whereas in forage from scrapie-free farms in scrapie-free areas manganese may be dominant over iron (ratio 1.09; not shown in Figure 6).

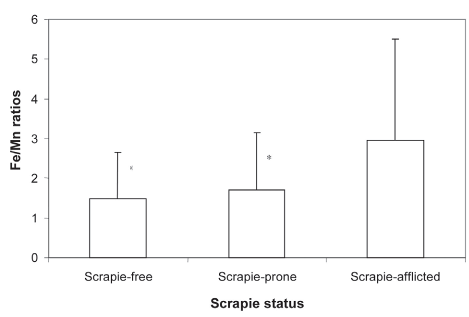

Figure 6. The calculated mean iron/manganese ratios in forage samples from 47 scrapie-free, scrapie-prone and scrapie-afflicted farms. Vertical bars show the standard deviation (from Gudmundsdóttir et al. 2006). * significantly lower ratio than in samples from scrapie-afflicted farms (P< 0.001, ANOVA).

Iron intoxication in plants seems to be a special problem when rice is cultivated on irrigated fields. Under these conditions the plant leaves may develop brown spots, or may even turn entirely brown, and the iron concentration in the leaves generally ranges from 300-1000 mg kg^{-1} (Mengel & Kirkby 1987). In our study the concentration of iron ranged from 500-1000 mg kg^{-1} in 14 samples (ca. 8%), indicating borderline cases of iron intoxication in the forage. A similar indication was also seen in the older results of Gudmundsson & Thorsteinsson (1980). The results of Gudmundsdóttir et al. (2006a), however, especially indicate that the high amounts of iron in the forage of sheep on scrapie-afflicted farms might somehow premise the occurrence of clinical scrapie through lower, albeit not deficient, levels of manganese.

In grazing animals iron deficiency is rarely seen and occurs primarily in animals infested with intestinal parasites. If the animals are fed on insufficient fodder, iron deficiency may occur, especially in calves fed solely on milk (Whitehead 2000). Iron intoxication in animals is seemingly only known after administration of inadvertently high doses of iron.

Iron-deficiency anaemia, which is the most common cause of nutritional anaemia in humans, may result from inadequate intake of iron to meet normal requirements, blood loss or some interference with iron absorption (Hillman 2001). Cases of iron intoxication occur, especially in children who, without purpose and foolishly, ingest large amounts of iron tablets (Goyer 1996). The incidence of such childhood iron poisoning is thought to be lower in Iceland than in the neighbouring countries.

DISCUSSION AND CONCLUSIONS

Iron is present in high abundance in forage on sheep farms in Iceland and it may even in some instances border on toxic levels in the plants.

This applies especially to samples from scrapie-afflicted farms. High amounts of soluble iron (or manganese) in plants can, especially in acidic soil, diminish the amounts of cobalt that are absorbed in the roots of plants. This might explain why the Co concentration in the feed of sheep in Iceland is on occasion lower than expected. Iron can also negatively affect zinc metabolism in plants, which might result in symptoms of zinc deficiency, as is occasionally seen in this country in cattle and sheep.

Although the high amounts of Fe^{2+} ions, as are often found in Icelandic soil, might presumably compete with the absorption of Cu^{2+} in plants, there are at the present time apparently no signs of copper deficiency in this country, neither in plants nor in sheep. This may in essence be explained by the high amounts of soluble copper present in Icelandic soil. Both zinc and molybdenum may have an adverse affect on copper metabolism. However, both of these metals occur in some cases in lower than optimal concentrations in the forage and may thereby indeed strengthen the copper status in the forage of sheep in Iceland.

There was no significant difference in the concentrations of cobalt, copper, molybdenum and selenium, respectively, in forage from farms in the various scrapie categories. On the other hand the amounts of manganese, iron and zinc did differ significantly in forage from farms in different scrapie categories. Manganese and iron moreover showed a spectacular reciprocality. Thus manganese was highest in forage samples from scrapie-free farms in scrapie-free areas where the iron concentration was lowest. In samples from scrapie-afflicted farms the reverse was the case. It can accordingly be assumed that high manganese can have a protective effect against the occurrence of clinical scrapie whereas high iron has a suppressive effect on the availability of manganese and at the same time a provocative effect on the occurrence of scrapie. It should be noted, however, that there were no signs of manganese deficiency observed in the sheep although the manganese concentration varied significantly between scrapie categories. It is also noteworthy that the zinc concentration was highest in samples from scrapie-free farms in scrapie-free areas where the iron concentration was lowest. Although little is known about the mechanism behind the presumed effect of certain trace elements in preventing or provoking clinical scrapie the results, referred to above, show beyond any reasonable doubt that concentrations of these elements in forage of sheep differ more or less in accordance with the scrapie status of the farms. In this context it is also of interest that the activity of the selenium enzyme glutathione peroxidase in the blood of non-pregnant ewes is higher on scrapie-free farms in scrapie-free areas than in ewes on farms in the other scrapie classes (Gudmundsdóttir et al. 2006b).

The normal prion protein (PrP^{c}) is secreted from the endoplasmic reticulum through the Golgi apparatus to the plasma membrane where it is tethered to the surface by a glycosylphosphatidylinositol anchor (GPI-anchor) (Cashman & Caughey 2004). The formation of the GPI-anchor in the endoplasmatic reticulum may involve glycosyl transferases that have a unique requirement for manganese as cofactor (Scott & Eagleson 1988, Kaufman et al. 1994). The normal prion protein (PrP^{c9}) is seemingly converted to the pathological form (PrP^{sc}) in near contact with PrP^{sc} at the membrane surface. This process appears to be catalyzed by specialized domains of the cell surface and the pathological protein is similarly bound to the cell surface by a GPI-anchor (Hicks et al. 2006). However, the pathological prion protein cannot be as easily cleaved from the GPI-anchor by a specific phospholipase as the normal protein (Stahl et al. 1990, Brown 2002).

High manganese concentration in the forage of sheep could hypothetically be instrumental in increasing the attachment of the prion proteins (PrP^{c} and PrP^{sc}) to cell membranes in the gastrointestinal tract and thus retard or prevent their entry through the border of the gastrointestinal mucosal epithelium, which is thought to be the main portal of entry of scrapie in the sheep (Brown 2003). This could perhaps explain the absence of clinical scrapie on scrapie-free farms in scrapie-afflicted areas. The present results could also indicate that zinc might be a part of this process. Since copper is known to facilitate the endocytosis of prion proteins it is to be expected that a copper chelator would delay the onset of prion disease in animal experiments (see copper). The results of further studies on soil and forage from sheep farms should primarily elucidate whether or not the high iron concentration present in forage on scrapie-afflicted farms is, as assumed, of paramount influence in contravening the apparent preventive effect of high Mn concentrations on the occurrence of sporadic scrapie.

The high concentration of iron in grass and forage of sheep in Iceland as demonstrated by Gudmundsson & Thorsteinsson (1980) and Gudmundsdóttir et al. (2006a) are seemingly in accord with the high levels of soluble iron in soils in Iceland (Sigvaldason 1992). High amounts of soluble iron in soils can decrease the availability of some other trace metals in plants resulting in disparate concentrations of the particular trace metals in the soils and the vegetation growing on the soils. Thus under Icelandic conditions, at least, studies on trace elements in soils and the relevant vegetation should preferably be performed concomitantly, whether they are aimed at research on scrapie or other diseases or the general health of domestic animals.

The work of Purdey (2000) deserves mention. This author determined manganese and copper in herbage (presumably leaves, stem and flowerheads from the upper half of the plants that sheep feed on in the free) in Svarfadardalur Valley, one of the scrapie-prone areas in northern Iceland (nr.4, Figure 1), and in an adjacent valley to the south that is known to be essentially free of scrapie. In the scrapie-prone valley herbage contained more manganese and less copper than in the scrapie-free valley. He therefore concluded that high amounts of manganese or low amounts of copper would favour the occurrence of clinical scrapie. In this context it should be noted that in Svarfadardalur Valley, although generally a scrapie-prone area, there were at the time of the study both scrapie-free, scrapie-prone and scrapie-afflicted farms within the valley. This is of fundamental importance as Jóhannesson et al. (2004a) have found significantly higher manganese concentrations in samples of forage from scrapie-free farms than from scrapie-prone or scrapie-afflicted farms in the same scrapie-affected areas. In this context it should also be emphasized that in Iceland there are few areas truly free of scrapie (scrapie never diagnosed, Figure 1).

Selenium deficiency is seemingly a ubiquitous phenomenon in sheep forage from cultivated plots in Iceland. The Se concentration is considerably higher in the wild mountain vegetation. Thus the Se status of the sheep is sufficient in the autumn, when the sheep are rounded up and gathered from the mountains, but it is at the deficiency level in the lambing season the following spring. White muscle disease is thus seen every spring in spite of prophylactic use of selenium as selenite injections. Selenium deficiency of this degree may have many deleterious effects on animal husbandry (sheep, cattle, horses) and should be abated by general measures as for instance Se supplementation to fertilizers, as has been practiced successfully in Finland for years (Eurola et al. 2005).

Trace elements that are essential in animal husbandry should permanently be a focal point for studies on the health of the animals. Thus the amount of a particular trace element present in animal feed may be insufficient to such an extent that it should be taken into account when planning wholesome feeding of the animals (e.g. selenium). On the other hand the utilization of certain trace elements may depend on the relative amounts of other trace elements present (e.g. the interference between copper and molybdenum and copper and zinc, respectively). In this connection it should also be reiterated that studies on a possible relationship between certain trace elements (iron, manganese and possibly zinc and copper) in the forage and symptoms of clinical scrapie in sheep are in the primary stage. In the future much more thoroughly performed studies in this field are therefore warranted.

The seven trace elements that are the topic of this presentation are certainly very important members of this group of elements. It would, however, have been desirable also to include in these studies the two other definitely important trace elements for livestock: chromium (Cr) and iodine (I). Little is known about chromium and iodine in Iceland. Thus it came as a surprise to diagnose sheep with classical symptoms of iodine deficiency (myxoedema in the face and struma) on pastures, seemingly swept by marine winds, not far from the southern coast. In line with this a preliminary study indicated a tendency, at least, towards deficient levels of iodine in the forage of sheep (unpublished results).

The results of determination of six essential macroelements (Na, K, Ca, Mg, P and S) in the same forage samples (170 in number) as were used for determination of the seven trace elements fell in the same range as the results of determination of these six macroelements in several hundreds of other forage samples collected during the same research period (2001-2003) (T. Eiríksson, unpublished results). The relatively small number of results obtained in our studies from determination of the seven trace elements may thus, we believe, reflect in the same way the general picture of these elements in the forage of sheep in Iceland in this period.

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