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Molybdenum and sulphur in forage samples from scrapie-free, scrapie-prone and scrapie-afflicted farms in Iceland

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ABSTRACT

It has been postulated by others that low levels of copper in herbage are connected with the occurrence of scrapie in Iceland. Although previous research by the authors does not support this idea the possibility might exist that molybdenum in the forage, along with high amounts of sulphur, could result in formation of copper chelates and thereby interfere with the bioavailability of copper and promote outbreak of clinical scrapie. Forage samples (in total 110) were collected on 36 sheep farms from the summer harvest in 2003 for analysis of molybdenum and sulphur. Farms were divided into three categories. *Scrapie- free*: Twenty-two farms never afflicted by scrapie, or afflicted and restocked with healthy sheep prior to 1960. *Scrapie-prone*: Seven farms afflicted by scrapie after 1980 and afterwards restocked with healthy sheep. *Scrapie-afflicted*: Seven farms where scrapie had been diagnosed during the experimental period (summer 2002 – March 2004). Results of copper analyses, formerly published by the authors, of the same samples from the summer harvest in 2003 as were used in the present study, were included for calculations of Cu/Mo ratios in the samples. Molybdenum concentration varied greatly in the samples, especially in those from scrapie-free and scrapie-afflicted farms. Sulphur concentration was remarkably constant in forage samples from farms in all categories. Cu/Mo ratios were most often in the range 10-100, the lowest ratio being 6. It was concluded that levels of molybdenum and sulphur in the forage are not directly connected to the occurrence of clinical scrapie and high individual Cu/Mo ratios indicate that molybdenum does not have a detrimental effect on copper status in Icelandic sheep.

Key words: Cu/Mo ratio, forage, molybdenum, scrapie, sheep, sulphur.

YFIRLIT

Mólýbden og brennisteinn í heysýnum á riðulausum bæjum, fjárskiptabæjum og riðubæjum á Íslandi

Sú tilgáta hefur verið sett fram að lítil koparþéttni í beitargróðri tengist uppkomu klínískrar riðu á Íslandi. Þótt fyrri rannsóknir höfunda styðji ekki þessa tilgátu, er sá möguleiki fyrir hendi, að mólýbden, ásamt mikilli þéttni brennisteins, geti hamlað nýtingu kopars vegna klóbindingar (chelation) í óvirk sambönd og þannig ýtt undir uppkomu riðu. Heysýnum úr rúlluböggum, alls 110 sýnum frá uppskeru sumarsins 2003, var safnað á 36 bæjum og mólýbden og brennisteinn ákvarðaður í sýnunum. Bæjunum var skipt í þrjá flokka: *Riðulausir bæir(“scrapie-free”)*: Tuttugu og tveir bæir þar sem riða hefur aldrei komið upp, eða riða greind og fjárskipti gerð fyrir 1960. *Fjárskiptabæir (“scrapie-prone”)*: Sjö bæir þar sem riða kom upp eftir 1980, og skipt var um fé. *Riðubæir (“scrapie-afflicted”)*: Sjö bæir þar sem riða kom upp á rannsóknartímabilinu (sumar 2002 – mars 2003). Áður birtar niðurstöðutölur ákvarðana á kopar í sömu heysýnum frá uppskeru sumarsins 2003 voru notaðar til þess að reikna kopar/mólýbden (Cu/Mo) hlutföll í sýnunum. Þéttni mólýbdens var mjög breytileg í heysýnum, einkum frá riðulausum bæjum og riðubæjum. Þéttni brennisteins var að meðaltali nær hin sama í sýnum frá bæjum í öllum flokkum. Cu/Mo-hlutföll voru oftast á bilinu 10-100 og hið lægsta var 6. Helstu ályktanir þessara rannsókna eru þær að 1) ekki virðast vera bein tengsl milli magns mólýbdens eða brennisteins í heysýnum og uppkomu riðu, og 2) há Cu/Mo í heyi hlutföll benda til þess að mólýbden hafi ekki ekki skaðleg áhrif á koparbúskap í fénu.

INTRODUCTION

The prion protein (PrP) is a normally encoded protein expressed in most organs. It occurs both as the free protein and as glycosylated protein bound to cell membranes. The prion protein contains copper and almost certainly has a role in copper metabolism and perhaps also in functions of the central nervous system and oxidative defense. In prion diseases (also called transmissible spongiform encephalopathies [TSEs]), of which scrapie in sheep and goats is one of the best known, the prion protein has taken on a misfolded and pathological form (most often referred to as PrP^{sc}) resulting in deposition of extracellular aggregates and spongiform changes (vacuolations) in brain tissue. These changes are accompanied by gliosis and neuronal death, and invariably, death of the animal. Although TSEs have many similarities to other amyloidoses, like Alzheimer´s disease, the unique and distinguishing feature of TSEs is that they alone are somehow transmissible between individuals of the same species or even between individuals of different species. However, most prion diseases have a sporadic occurrence, suggesting the presence of environmental factors, like metals, that may influence the conversion of PrP to PrP^{sc} or otherwise influence the pathogenicity of the PrP^{sc} protein (Prusiner 2001, Brown 2002, Brown 2003, Jóhannesson et al. 2003). This idea has been the focus of our previous studies on manganese, copper, selenium and scrapie (Jóhannesson et al. 2004a, 2004b). In the present study the scope of these experiments has been extended to include molybdenum and sulphur.

In the presence of ample amounts of sulphide, as is found in the rumen of sheep or cattle, molybdenum is transformed, to a lesser or greater degree, into tetrathiomolybdate (or other thiomolybdates). These compounds have a strong copper-chelating activity and can interfere with the bioavailability of copper in the gastrointestinal tract of ruminants. This is especially likely to occur if the Cu/Mo ratio in the feed is lower than 4 (Adriano 2001). High levels of molybdenum in the forage of sheep, and especially if accompanied by high levels of sulphur, might thus have a negative influence on copper metabolism in the sheep and render support to the postulate of Purdey (2000) that low levels of copper favour the occurrence of clinical scrapie.

Molybdenum has, to our knowledge, never been analysed in the forage of sheep in Iceland and the results of earlier sulphur determinations in forage in Iceland are fragmentary. It is accordingly unknown whether differences in the amounts of molybdenum or sulphur in the feed of sheep might have any relevance to the occurrence of clinical scrapie in Iceland. In the present study we have therefore analysed molybdenum and sulphur in forage samples from the 2003 harvest on farms in all three scrapie categories, as described by Jóhannesson et al. (2004b). In order to study specifically the Cu/Mo relationship in the samples we have also included results of the copper analyses from the 2003 harvest (Jóhannesson et al. 2004a).

MATERIALS AND METHODS

Categories of farms and collection of hay samples

Forage samples, mainly from round-bale silage, were collected on 36 farms from the summer harvest in 2003. The total number of samples was 110. The farms were divided into three categories as previously described by Jóhannesson et al. (2004b). *Scrapie-free*: Twenty-two farms never afflicted by scrapie, or afflicted and restocked with healthy sheep prior to 1960. *Scrapie-prone*: Seven farms afflicted by scrapie after 1980 and afterwards restocked with healthy sheep. *Scrapie-afflicted*: Seven farms where scrapie had been diagnosed from summer 2002 to March 2004. The farms are situated in the same 11 localities in southern, western and northern parts of the country as described by Jóhannesson et al. (2004a).

Samples were taken of grass silage (from 30-70% dry matter) from the cultivated home fields and preferably from older and more recently cultivated plots, respectively, as previously described by Jóhannesson et al. (2004a). About three different grass silage samples (two to five) were collected on each farm. In a few instances samples consisted of fully dried hay (≥85% DM) as a few farmers still adhere to the older practice of hay making. For convenience all samples are referred to as *forage*, whether fully dry (traditional hay) or only partially dry (grass silage).

Preparation of samples and analyses of molybdenum and sulphur

All samples were dried at 65 °C in a forced air oven for 48 hours. After being stabilized at room temperature samples were milled through a 1 mm screen. Milled samples were weighed for molybdenum analyses and analyses of sulphur and at the same time weighed to estimate sample dry matter. Samples were then digested by boiling in concentrated HNO_3 (Merck, Suprapur) overnight and subjected to analysis. Analyses for S were carried out by ICP optical emission spectrometry using a Spectroflame D sequential instrument (Spectra, Germany). Analyses of Mo were carried out on the above-mentioned nitric acid digests by graphite furnace atomic absorption spectrometry with erbium nitrate as a matrix modifier, using an Hitachi Z-8300 instrument with Zeeman effect background correction.

Three analytical samples (parallels) were taken from each forage sample. The results of individual forage samples were, respectively, the means of measurements of the three analytical samples and are expressed as mg kg^{-1} dry matter for molybdenum or g kg^{-1} dry matter for sulphur. Several samples of a certified reference material (Leaves of Poplar NCS CC 73350) were taken through the analytical procedures to confirm accuracy of measurements.

Statistical analyses

The Kolmogorov-Smirnov test was used to test for normal distribution. One way analysis of variance (ANOVA) was used for comparison of normally distributed analytical results (sulphur concentrations). The Kruskal-Wallis test was used for comparison of results that were not normally distributed (molybdenum concentrations).

RESULTS

Individual intra-sample results did not differ more than ± 5%. Molybdenum concentrations varied from zero to 1.37 mg kg^{-1}. In forage samples from the 22 scrapie-free farms the average concentration was 0.24 mg kg^{-1}. In samples from the seven scrapie-prone farms the average concentration was 0.13 mg kg^{-1} and in forage samples from the seven scrapie-afflicted farms, 0.23 mg kg^{-1}. The collective distribution of individual results of molybdenum determinations in forage samples from scrapie-free, scrapie-prone and scrapie-afflicted farms, respectively, is shown in Figure 1. The distribution of individual results was wide for samples from the scrapie-free and scrapie-afflicted farms and did not pass the Kolmogorov-Smirnov test for normality. Distribution of individual results was far less for samples from scrapie-prone farms and the results were normally distributed. When subjected to the Kruskal-Wallis test, nevertheless, no significant difference was found between the groups (P > 0.05).

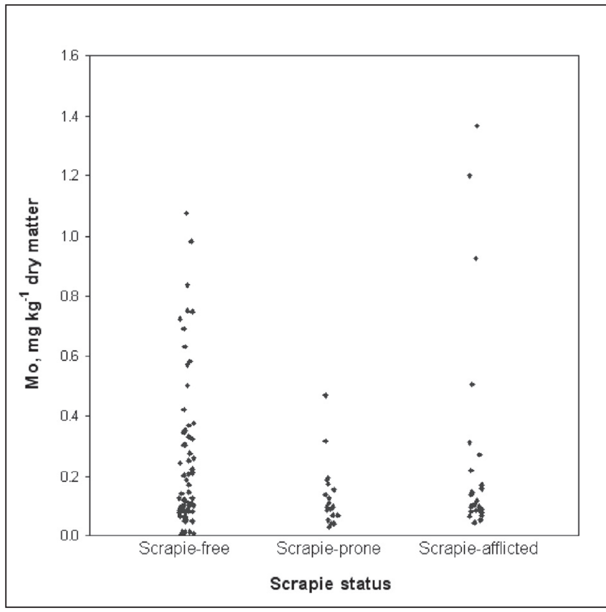

Figure 1. Collective distribution of individual results of molybdenum determination in forage samples from scrapie-free, scrapie-prone and scrapie-afflicted farms.

For the scrapie-free farms the mean value for the Cu/Mo ratio was 116.3 (range 8.8-1020, median 58.1). For the scrapie-prone farms the mean Cu/Mo ratio was 110.8 (range 14.5-274.8, median 80.3), and for the scrapie-afflicted farms the mean Cu/Mo ratio was 73.2 (range 5.9-160.2, median 71.2). Individual Cu/Mo ratios thus varied widely from 5.9 to 1020. In all categories most Cu/Mo ratios (about 60-70%) were in the range of 10-100. Some ratios were in the range of 100-200 and a few were still higher. There did not seem to be a connection between molybdenum concentration (or Cu/Mo ratio) and the age of the sampled plots.

Sulphur concentrations varied about fivefold from 0.88 g kg^{-1} to 4.2 g kg^{-1}. The average concentration of sulphur was almost the same in samples from farms in all categories (2.34 g kg^{-1} on scrapie-free farms, 2.36 g kg^{-1} on scrapie-prone farms and 2.29 g kg^{-1} on scrapie-afflicted farms). The results were normally distributed for farms in all categories. No significant difference was found between the groups when subjected to one way analysis of variance (P > 0.05).

DISCUSSION

The "normal" concentration of molybdenum for plants can vary widely or from 0.01 to 1.0 mg kg^{-1} or more according to the plant species (Frøslie et al. 1983, Adriano 2001). Most of the results presented here are therefore within the normal range (Figure 1).

The range of individual molybdenum concentrations was lowest (approximately 0.05 - 0.5 mg kg^{-1}) in samples from scrapie-prone farms and these concentrations were normally distributed. In samples from scrapie-free farms and scrapie-afflicted farms the concentration of molybdenum was most often in the same range. In these categories, however, individual concentrations were, on some occasions, above 0.6 mg kg^{-1}, resulting in a skewed distribution and higher values of average concentrations (Figure 1). The Kruskal-Wallis test did not reveal any significant difference between the three categories.

In most instances the calculated Cu/Mo ratio was found to be in the range of 10-100. It was lower in a few instances and in a few samples ratios higher than 200 were also found. It is well known that molybdenum, in the presence of ample sulphide as in the rumen of sheep and other ruminants, may form tetrathiomolybdate or other thiomolybates that actively chelate copper and may even be absorbed and interfere with copper availability inside the body (Frøslie et al. 1983, Adriano 2001, Spears 2003). The general consensus is, however, that the critical level for occurrence of these phenomena is when the Cu/Mo ratio is lower than 4 (Adriano 2001). In our forage samples the lowest individual Cu/Mo ratio was about 6. It can therefore be assumed that the amounts of molybdenum found in forage available to sheep in Iceland are not detrimental to copper metabolism in the sheep. These results support the contention that there are no obvious signs of copper deficiency in sheep in Iceland (Jóhannesson et al. 2005).

Purdey (2000) found that low concentrations of copper in herbage (or high concentrations of manganese) were connected with the occurrence of clinical scrapie in Icelandic sheep. This contention is not supported by our previous work on manganese and copper in forage samples from farms in different scrapie categories (Jóhannesson et al. 2004a). Neither are the results presented here in support of Purdey´s (2000) idea as the Cu/Mo ratios in samples from farms in all categories were well above the critical level for a detrimental effect of molybdenum on the bioavailability of copper in the feed of sheep.

Sulphur concentration was on average about the same (approximately 2.3 g kg^{-1}) in forage samples from scrapie-free, scrapie-prone and scrapie-afflicted farms, and the individual results were normally as well as equally distributed. Thus the results indicate that forage sulphur status is the same on farms in all categories. Levels of sulphur were also in the same range in a large number of forage samples (nearly 2200) collected from the 2004 harvest on farms in the southern and western part of the country (average: 2.24 g kg^{-1} ± 0.048 s.d.) and the northeastern and eastern part of the country (average: 2.35 g kg^{-1} ± 0.045), respectively (unpublished results). Therefore the levels of sulphur in the forage seem to be remarkably constant in Iceland.

The sulphur requirement for optimal animal growth varies, ranging from 2.0 g kg^{-1} to 5.0 g kg^{-1} of plant dry weight (Wu et al. 2003). Most of the results presented here are above 2.0 g kg^{-1} and only very few are lower than 1.5 g kg^{-1}. Accordingly it can be assumed that the amounts of sulphur in forage in Iceland are adequate. Although sulphur may interfere with the availability of selenium (Adriano 2001) it is doubtful whether these levels of sulphur are in any way connected with the widespread selenium deficiency found in forage of sheep in Iceland (Jóhannesson et al. 2004b).

In summary, the results show that individual concentrations of molybdenum vary highly in forage of sheep in Iceland while the concentrations of sulphur are remarkably constant. Differences in molybdenum concentrations in forage samples are not connected to differences in scrapie status, nor do the high Cu/Mo ratios found indicate any detrimental effect of molybdenum on copper in the sheep.

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REFERENCES

Adriano AC 2001. *Trace elements in terrestrial environments. Biogeochemistry, bioavailability, and risks of metals*. Chapter 15 (molybdenum). Springer, 2nd ed. 867 p.

Brown DR 2002. Conclusion: Future directions for copper and the prion protein. In: Brown DR (ed.) *Prion Diseases and Copper Metabolism, BSE, Scrapie and CJD Research*. Horwood Publishing, Chichester pp. 187-194.

Brown P (with contributions of others) 2003. *Transmissible Spongiform Encephalopathy as a Zoonotic Disease*. ILSI Europe Report Series. 48 pp.

Frøslie A, Ulvund MJ, Maina JN & Norheim G 1983. Trace elements in grass, cattle liver and sheep liver from districts surrounding Karatina, Kenya, II. Copper, molybdenum, zinc and sulphur. *Nord. Vet.- Med*. 35, 213-218.

Jóhannesson T, Kristinsson J & Snaedal J 2003. Hrörnunarsjúkdómar í heila – oxavarnarensím og kopar. Kynning á rannsóknum. (Neurodegenerative diseases, antioxidative enzymes and copper. A review of experimental research). *Læknablaðið* 89, 659-671. (In Icelandic, English summary).

Jóhannesson T, Gudmundsdóttir KB, Eiríksson T, Kristinsson J, Sigurdarson S 2004a. Copper and manganese in hay samples from scrapie-free, scrapie-prone and scrapie-afflicted farms in Iceland. *Icel. Agric. Sci*. 16-17, 45-52.

Jóhannesson T, Gudmundsdóttir KB, Eiríksson T, Barash J, Kristinsson J, & Sigurdarson S 2004b. Selenium and GPX activity in blood samples from pregnant and non-pregnant ewes and selenium in hay on scrapie-free, scrapie-prone and scrapie-afflicted farms in Iceland. *Icel. Agric. Sci.* 16-17, 3-13.

Jóhannesson T, Gudmundsdóttir KB, Barash J, Kristinsson J, Eiríksson T, & Sigurdarson S 2005. Manganese, copper and copper enzymes in blood of Icelandic sheep: Relevance to scrapie *Icel. Agric. Sci*. 18, 33-42.

Purdey M 2000. Ecosystems supporting clusters of sporadic TSEs demonstrate excesses of the radical-generating divalent cation manganese and deficiences of antioxidant cofactors Cu, Se, Fe, Zn. Does a foreign cation substitution at prion protein's Cu domain initiate TSE? *Med. Hypotheses* 54, 278-306.

Prusiner SB 2001. Shattuck lecture – Neurodegenerative diseases and prions. *N. Engl. J. Med.* 344, 1516-1526.

Spears JW 2003. Trace metal bioavailability in ruminants. *J. Nutr.* 133, 1506S-1509S.

Wu L, Guo X & Banuelos G 2003. Selenium and sulfur accumulation and soil selenium dissipation in planting four herbaceous plant species in soil contaminated with drainage sediment rich in both selenium and sulfur. *Int. J. Phytorem.* 5, 25-40.

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