*Netherlands Journal of Agricultural Science 41 (1993) 47-57*

# Liver selenium analysis in cows with a fast method of neutron activation reveals deficiency areas in the Netherlands

W. T. BINNERTS[1], H. A. DAS[2] & T. C. VIETS[1]

[1] Department of Human and Animal Physiology, Wageningen Agricultural University, Haarweg 10, NL 6709 PJ Wageningen, Netherlands
[2] Netherlands Energy Research Foundation, Petten, Netherlands

Received 24 February 1992; accepted 7 January 1993

## Abstract

A new method has been used for analysis of selenium in cow's liver. It consists of accurately timed 5 seconds irradiation with neutrons in a high flux generator, immediately followed by measurement of the short-lived isotope $^{77m}$Se in a rigid time sequence, under fully automated conditions. Thus 230 freeze dried liver samples were analysed. They were obtained from well-defined origins at 13 Dutch slaughterhouses. The liver selenium contents ranged from 0.16 to 1.82 mg/kg dry matter. In the total material no relation to geography could be demonstrated.

Some 3 % of the livers contained less than 0.25 mg/kg dm, considered critical for development of grave selenium deficiency. Another 20 % with less than 0.4 mg/kg dm could be termed borderline selenium deficient. There was no evidence in this material of toxicity of selenium. The frequency distribution of the results was not uniform, but after logarithmic transformation it could be separated into two sets, both normally distributed. The liver selenium content had a positive correlationship with liver copper, which however, may be circumstantial, and derived from the occurrence of Se and Cu in the feed. With liver zinc contents there was not such a relation. Further study revealed that the two distributions of the frequency diagram may be related to feeding management. The lower group most probably relates to non-lactating animals, with limited or no access to concentrates. In this group some relation of liver Se with soil types could be observed. The other, higher and larger, group then are the milk cows, with currently large amounts of concentrates fed in addition to roughage. It seems that this group due to the high selenium content of imported concentrates, is well cared for. On the other hand, young animals, especially heifers, frequently have an unsatisfactory, low, Se-status.

*Keywords:* liver selenium content, fast analysis, neutron activation, selenium deficiency, cattle

## Introduction

Selenium is an essential trace element, required to maintain health in man and animals, with effects on growth and (re)production (Levander, 1986; Sunde, 1990). A number of selenium deficiency conditions, retained placenta, degeneration of muscle,

liver, and pancreas, seems to be the result of peroxidative tissue damage. Other conditions, like unthriftiness of sheep and cattle, may relate to insufficient thyroid expression and/or immune function. This is in line with the knowledge that selenium is a structural part of two important enzyme systems: the glutathione peroxidases and type I-deiodinases, needed for neutralizing potentially toxic peroxides of metabolism, and for generation of active thyroid hormone (Behne et al., 1991). The Se-requirement in absolute sense, is to a certain extent variable, due to positive (vitamin E) and negative factors (heavy metals).

In the Netherlands low and higher selenium regions have been described after milk and blood analysis (Binnerts, 1979). Higher levels were found in areas with marine and river silt (clay) and lower levels in sandy and peaty areas, suspected to be more or less Se-deficient. The latter was confirmed by analysis of blood, for young animals (Counotte & Hartmans, 1990) and in study on cases of retained placenta (Wentink et al., 1988). It is quite well possible that also a number of other ailments related to immunity problems exist in these regions for which it is difficult to prove that they are caused by selenium deficiency (Stevenson et al., 1990). The availability of a reliable and fast method of selenium analysis (Woittiez & Nieuwendijk, 1987) offers good possibilities for further research. Up to now the selenium analysis has been difficult and an obstacle in this respect. Milk has been analysed by neutron activation in the classical, time consuming, way (Binnerts, 1979) and also by atomic absorption spectroscopy coupled to hydride generation (Binnerts et al., 1984). The latter method in our hands however was found insufficient for liver samples, recently giving too low recoveries. Among the possible substrates (blood, cells, milk, tissues) the liver has been chosen, because it reflects the selenium status relative to all types of selenium (inorganic as well as organic) in the feed (Butler et al., 1990, Lane et al., 1991, Behne et al., 1991) and because it can be easily obtained in quantity, and from well-defined origins.

The purpose of this study is to investigate the selenium status of cattle in the Netherlands. At the same time experience was gained in selenium analysis by the fast neutron activation method in biological samples, which we wish to bring to focus for agricultural research purposes.

## Materials and methods

A total of 230 liver samples were obtained at 13 slaughterhouses in the Netherlands at the end of 1982. This is the most recent collection of liver samples from which the copper (Binnerts, 1986) and zinc contents (Binnerts, 1989) have been reported, but for 12 samples which had been omitted earlier from this set for technical reasons. The samples were all from the caudal lobe, some 100 g in weight, and they were packed in polythene bags and stored at -20 °C until use. Subsamples were taken, weighed, cut into small pieces, freeze dried, powdered and weighed again into 16.75 × 8.0 mm (internal dimensions) high purity polythene vials with snap cap, which fit into 28.6 × 14.9 mm (external dimensions) shuttles of the same material.

Irradiation was performed in the automatic so-called FASY rabbit system in the high flux reactor (HFR) at Petten, the Netherlands (Woittiez et al., 1987, 1988),

Fig. 1. Sampling and the origin of over 200 livers. (Some samples have been omitted, which could not be accurately traced; these include the 12 additional samples (see text) and 5 samples east and 5 samples south of Leeuwarden, the most northernly situated sampling station). Sampling stations are represented by black squares.

which can be loaded with up to 100 aliquots. Irradiation time was 5 s at a thermal neutron flux of $4.10^{13}$ $cm^{-2}$ $s^{-1}$. Return to the counting position took about one second, during which capsule and shuttle were separated; the capsule arrived in the counting position. After a decay time of 5 s, counting followed for 20 seconds. An intrinsic Ge detector of 35 $cm^3$ volume was used, connected to a 4000 channel analyzer at 0.5 keV per channel. The 161.9 keV photopeak of $^{77m}Se$, t½= 17.5 s, was evaluated by linear interpolation of the compton background. Correction for residual dead-time losses were based on the continuous recording of the total dead-time fraction over the measuring period (Nieuwendijk et al., 1985). The relative standard deviation due to counting statistics was calculated according to Das et al., 1979. The net specific

count rate under the 161.9 keV photopeak was $1.83.10^4 \pm 3.10^2$ c (μg Se)$^{-1}$. All computations were performed by the dedicated microcomputer which controls the apparatus. The results of earlier copper, zinc, and iron analysis were available (Binnerts, 1986, 1989).

The location of slaughterhouses and samples is given in Fig. 1. Most of the samples originate from a rather narrow area around the slaughterhouses. The 12 samples, not used in the earlier copper and zinc studies have not been indicated in Fig. 1; they are fairly well spread around the close vicinity of the slaughterhouses: Arnhem (three times), Den Bosch (three times), Soest (three times) Sittard, (one time), Zevenaar (one time), and Leeuwarden (one time).

The results were tabulated and statistically treated (Lotus, and SPSS package).

## Results

The added reference sample (NBS liver sample reference SRM 1577a) with a certified selenium content of 0.71 mg/kg dry matter (dm) was found to have 0.70 ± 0.045 mg/kg dm in sixfold analysis. The 230 results from the liver investigation ranged from a low 0.16 up to 1.83 mg/kg dm. The average of these results was 0.67 ± 0.25, and the median just over 1 mg/kg dm.

As will be seen in Table 1, in the first column (the latter two columns will be discussed in Section c of the Discussion) the regions of Sittard, and the sub-areas of Friesland, Sneek and Drachten, are low in selenium, whereas Arnhem and the subgroup Leeuwarden are amongst the highest. The differences from the mean, however are not significant, owing to the large amount of variation within the groups ($P > 0.05$). Very low single values came from the area of Sittard (0.159) and Soest (0.185), and rather high ones from Den Helder (1.70) and Leeuwarden (1.83), all values being expressed as mg/kg dm of liver tissue. The wide span of the results, in relation to the often-suggested narrow band separating deficient and toxic selenium

Table 1. Regional average selenium content in liver (mg/kg dry matter).

| Region | All samples | Concentrate-fed | Non-concentrate fed |
|---|---|---|---|
| Arnhem | 0.77 ± 0.22[1](21)[2] | 0.82 ± 0.19 (18) | 0.49 ± 0.05 (3) |
| Den Bosch | 0.73 ± 0.22 (29) | 0.79 ± 0.19 (24) | 0.44 ± 0.03 (5) |
| Soest | 0.61 ± 0.38 (20) | 0.78 ± 0.36 (13) | 0.28 ± 0.06 (7) |
| Sittard | 0.50 ± 0.22 (22) | 0.70 ± 0.27 (7) | 0.40 ± 0.11 (15) |
| Alkmaar/Den Helder | 0.75 ± 0.35 (34) | 0.92 ± 0.36 (22) | 0.43 ± 0.13 (12) |
| Leiden | 0.71 ± 0.19 (20) | 0.77 ± 0.14 (16) | 0.47 ± 0.14 (4) |
| Meppel | 0.54 ± 0.25 (8) | 0.67 ± 0.23 (5) | 0.32 ± 0.05 (3) |
| Zevenaar | 0.66 ± 0.19 (13) | 0.77 ± 0.16 (7) | 0.54 ± 0.14 (6) |
| Venray | 0.67 ± 0.28 (15) | 0.76 ± 0.27 (11) | 0.44 ± 0.16 (4) |
| Friesland[3] | 0.68 ± 0.42 (32) | 0.93 ± 0.40 (18) | 0.37 ± 0.16 (14) |
| Texel | 0.59 ± 0.29 (16) | 0.81 ± 0.33 (7) | 0.48 ± 0.12 (9) |
| Total | 0.67 ± 0.31 (230) | 0.82 ± 0.29 (148) | 0.41 ± 0.13 (82) |

[1] Standard deviation. [2] Number of samples. [3] Total of Leeuwarden, Sneek and Drachten, which are separately in the first column: 0.78 ± 0.45 (21); 0.48 ± 0.28 (5) and 0.53 ± 0.39 (6).

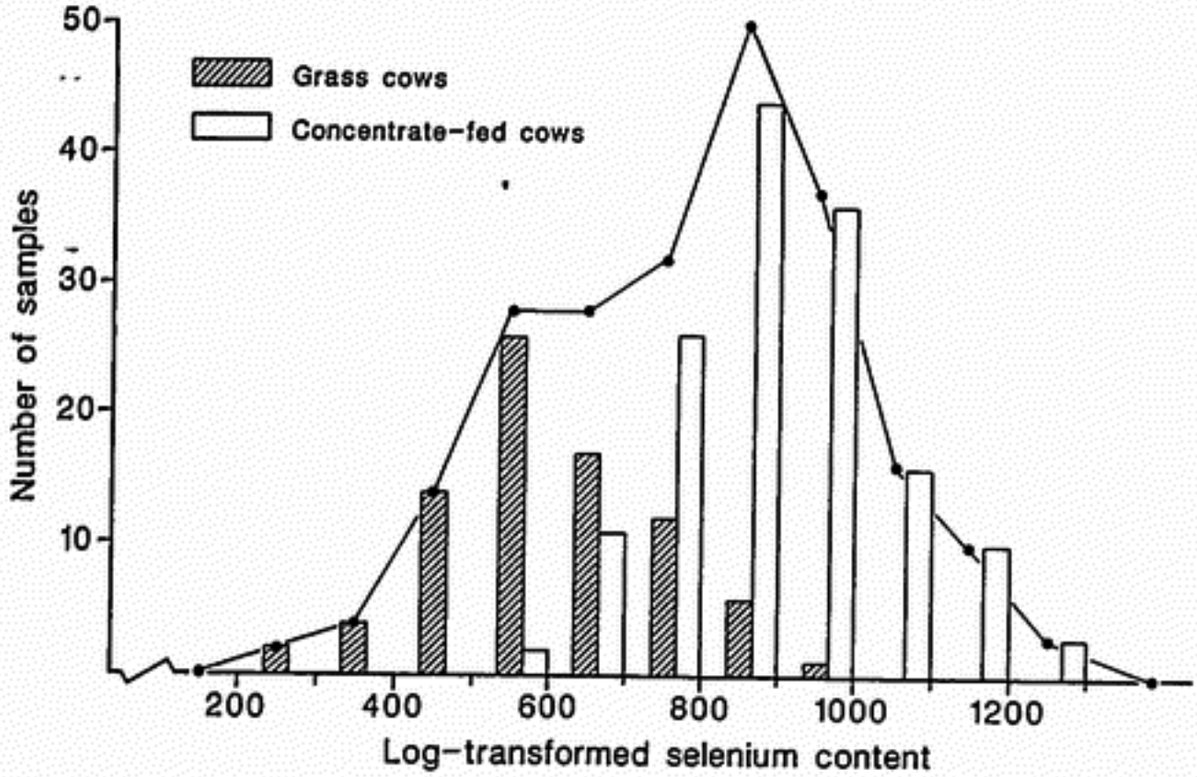


Fig. 2. Frequency distribution of selenium in liver samples in the Netherlands, with normal distributions. (The transformation is made by expressing the selenium content in ng/g dm, and by taking 1000 (log Se-2) in classes of 100. In order to adjust to the real average value a further normalisation factor of 667/824 should be applied). The normal blockwise representation gives the two uniform distributions (Discussion, Section c). The drawn line connects the midpoints for similar blocks of the total set of values, which clearly has a complex distribution. This unorthodox way of presentation of the total group is chosen to avoid confusion by overdrawing. For the same reason the original liver selenium values in mg/kg dm have not been indicated. Here we give some of them: 0.25 for 400, 0.40 for 600, 0.80 for 900, and 1.60 for 1200 log scale.

contents, gives rise to some concern (see Discussion). The frequency distribution of the results (Fig. 2) even after log-tranformation, was not uniform. It could be separated into two uniform ones, including a zone of overlap. In Fig. 2 these distributions have been indicated. They most probably relate to two types of management and feeding conditions, which will be discussed in the next paragraph.

## Discussion

Although there is a general consensus in literature on the fact that tissue concentration of selenium will increase with increasing Se intake, and that serum and liver Se provide good indicators of dietary Se status in cattle (McDowell et al., 1990) there remains a need for better understanding and precision. Therefore some further study has to be made, and the following subjects seem to be important for the interpretations of our results: a) the time of sampling in relation to especially b) the characteristics of the liver selenium metabolism, and last but not least c) the selenium content of the feed as related to farm management. After discussion of the value of the here given liver selenium contents, some side-effects relating to d) interaction of Se with

Cu, Zn, etc. will be discussed, and finally some thoughts will be devoted to e) sampling of blood and milk versus liver, and f) the prevention and treatment of selenium deficiency in cattle.

*a. Time of sampling*

The time of sampling in this research had been devised mainly for the copper analysis (Binnerts, 1986). It was the last week of October and the following weeks of November, to allow for a good survey of the whole grazing period. In the presentation of the zinc values (Binnerts, 1989) it has already been remarked that for zinc this period of sampling would cover only the last part of the summer season, because zinc is much more mobile than copper in the liver metabolism. Now, with selenium, we come to the other extreme; there is hardly any stapling, and the liver content reflects only the selenium status of not much more than just a few weeks. Therefore two periods are reflected; the very end of the grazing time, and the start of the stallfeeding. One could therefore call the groups pasture fed versus stall-fed. This will be elaborated further in this Section. A final question is, do we have to repeat this research some 25 times over the year in order to obtain an impression of the year-round Se status? Probably not, although the period of overview by this liver Se contents is just a few weeks, the expected variation over the months will be small (McDowell et al., 1990) compared to the large difference of pasture versus stall feed content (Section c). Some repetition, of course would be helpful.

*b. Characteristic selenium metabolism of the liver*

This metabolism is responsible for the short period of overview, just mentioned. The liver is, with the kidney, among the body tissues with the highest selenium content (Levander, 1986), and it is the fastest in contribution to and equilibrating with the total body selenium and the selenite-exchangeable metabolic pool (Janghorbani et al., 1991), regardless of past selenium intake, chemical form of selenium or age and size of the animals. Undoubtedly this has to do with the intensive metabolism and ability to synthesize and secrete specific seleno-proteins for export by the blood to other body compartmens (Reiter et al., 1989).

*c. Selenium content of the feed*

Our sampling is relevant for the selenium status of cows around 1 November. The year of sampling, 1982 or today is not relevant, because the management has not changed greatly in the latest decade. The collection at slaughterhouses has the usual drawback, in that it is not representative for the population of living animals. However, at the end of the grazing season this drawback is minimal, since at that time not only the less satisfactory cows are being sent to slaughter, but cows in all age groups, that cannot be maintained in the limited space of winter stalls. They will be discharged in addition to the planned number of 'fattened' (Dutch: 'vetweiders') or meat cattle.

For comparison of the observations with literature little information on liver selenium values is available (Table 2). The values in the table apart from ours stem from Denmark, Canada, Norway and the USA.

The value of 0.25 mg/kg dm has been given by McDowell, 1985, as low critical value for cows in tropical conditions, with sufficient vitamin E supply, and probably not suffering from any important heavy metal ingestion. In using it, Prabowo et al., 1990, found deficiency in only 9 % of 200 liver samples from Sulawesi (former Celebes), Indonesia. (In our study for the Netherlands it would have been 6 cases out of the total 230; less than 3 %). Such cases have a definite chance of grave deficiency with symptoms, including muscle and liver degeneration. This is expressed by the term CC: critical concentration level. Actually, also a critical level of somewhere between 0.25 and 0.50 mg/kg dm has been proposed (McDowell et al., 1990). Froeslie et al., 1980, with a low borderline value of 0.2 mg/kg fresh liver materials (approximatively 0.6 mg/kg dm) came to 88 % deficiency for cattle in Norway. As a borderline deficiency value for the Netherlands we would like to propose 0.40 mg/kg dm, which is based on our experience. It can be found in Fig. 2, for the lower side of the left frequency distribution. With this lower value 50 % or so of the cows in the lower group – pasture fed – would be in danger for some type of selenium deficiency. McDonnell mentions to this effect a boundary value of 0.05 mg/kg dm in the feed (McDonnell et al., 1990). For a wider group of feeds and feed ingredients we have composed Table 3.

As will be seen from the table the average content of most of the roughages in our area is lower or equal to the boundary value of 0.05 mg/kg dm, but additions to the ration of small amounts of concentrates give a large improvement. For milk producing cows, stall-fed or even on pasture the trend of the last decades has been to supply much additional concentrate. With the imported soya in our regions this will even lead to rather high selenium supplies. Hence two different groups of cows have been created, relative to selenium intake; the roughage group (mostly dry cows) with feed selenium content around and below 0.05 mg/kg dm, and the group fed additional concentrates (mostly milk producing cows) with average feed selenium well above 0.1 mg/kg dm. On this basis, as an exercise, we have prepared the two last columns of table 1, and the groups of Fig. 2. Since no information was available on the parameter dry/milk production (this had not been considered necessary in the previ-

Table 2. Liver selenium content in literature, expressed in mg/kg[1]

| Year | Author | Species | Number | Low | Medium | High |
|---|---|---|---|---|---|---|
| 1970 | Bisbjerg | cow | 10 | | 0.7[d] | |
| 1980 | Korsrud | cattle etc. | 600 | | | 1.9[d] |
| 1980 | Froeslie | cow | 212 | | 0.14[f] | |
| 1985 | McDowell | cow | | 0.25[d] | | |
| 1987 | Norheim | pig, man | | | 0.44[f] | |
| 1992 | this report | cow | 230 | 0.16[d] | 0.67[d] | 1.83[d] |

[1] With f for fresh, d for dry materials. For intercomparison: d values are about three times f values, except for biopsy and autopsy, when the factor can be 4-5 by residual blood content.

Table 3. Selenium content of feeds and feed components (mg/kg dm).

| Material | Selenium content | Author | Year | No of samples |
|---|---|---|---|---|
| Grasses | 0.016 | Froeslie | 1980 | 40 |
| Hay | 0.05 | our results | 1985 | 4 |
| Barley, oats | 0.0009 | Froeslie | 1980 | 63 |
| Barley | 0.069 | Schulte cs | 1987 | 3 |
| Vegetables | 0.05 | Schulte cs | 1987 | 44 |
| Rapeseed meal | 0.55 | Froeslie | 1980 | 13 |
| Rapeseed meal | 0.154 | Schulte cs | 1987 | 2 |
| Soybean meal | 0.49 | Froeslie | 1980 | 17 |
| Soybean products | 0.47 | Schulte cs | 1987 | 2 |
| Tapioca | 0.11 | Schulte cs | 1987 | 1 |
| Concentrates | 0.22 | Froeslie | 1980 | 45 |
| Concentrates | 0.15 | Netherlands* | 1980 | 15 |
| Concentrates | 0.19 | our results | 1985 | 5 |

* Netherlands Mineral Nutrition Commission, personal communication.

ous research) the separation of the two groups was entirely made on basis of the liver selenium content, aided for the overlap zone by the copper content (For most of the grazing cattle the liver copper stores become exhausted towards the end of the season). It will now be seen in the last column of table 1, that the groups 'non-concentrate fed' had much lowered standard deviation, permitting conclusions based on geography. This was not evident in the total material (column 1). The groups of Meppel and Soest would be low in selenium nearing the critical level at 0.3 mg/kg dm Obviously the large import of concentrates obscures the geographical distinctions, at the same time bringing the selenium supply of milk cows to safe levels. Some higher liver-Se contents in this group stay well below 2 mg/kg dm, and in our opinion cannot yet be termed toxic.

*d. Interrelationship copper, zinc and iron*

The liver selenium and copper in this work have a positive correlation ($r = 0.50$). Probably this correlation is circumstantial, and induced by the pairwise high copper and selenium levels in some concentrates, and the general low level in roughages. Further no relationships have been traced, apart from the following suggestions (Table 4). Livers with much iron had on the average unaffected copper, zinc and selenium contents. Some individual cases had lowered Cu (Cu deficiency induced iron staple) and also somewhat lower Se.

High copper contents tended to accompany somewhat elevated Se and Zn. Low and high Se content had no relation with Zn, but some binding to Cu. Increased zinc accompanied higher Cu, but unchanged Se.

Low zinc accompanied both low Cu and Se. The relation with zinc has also been remarked in induced copper deficiency (Fields et al., 1984) whereby fructose feeding to rats Cu and Se-enzymes decreased and liver Cu and Se decreased, indicating a

Table 4. Interactions of selenium, copper, zinc and iron as witnessed by the liver contents (mg/kg dm, except for Se: µg/kg dm). The elements are represented by their symbols. The contents are given with the standard deviations, and the number of samples (in brackets). The positive and negative shifts are symbolized by arrows.

| | | | |
|---|---|---|---|
| ↑ | High Se (concentrate group) | ↓ | Low Se (roughage group) |
| ↑ | Cu 292 ± 166 (148) | ↓ | Cu 84 ± 112 (82) |
| - | Zn 156 ± 81 | | Zn 145 ± 59 |
| ↑ | High Cu (over 400) | | Normal and low Cu |
| ↑ | Se 926 ± 347 (39) | | Se 619 ± 277 (191) |
| ↑ | Zn 197 ± 127 | | Zn 143 ± 53 |
| ↑ | High Zn (over 250) | | Normal and low Zn |
| | Se 675 ± 256 (21) | | Se 677 ± 319 (197) |
| ↑ | Cu 332 ± 256 | | Cu 210 ± 165 |
| ↓ | Low Zn (below 100) | ↑ | High Fe (over 400) |
| ↓ | Se 473 ± 170 (48)) | | Se 588 ± 172 (17) |
| ↓ | Cu 162 ± 99 | ↓ | Cu 172 ± 158 |
| | | ↑ | Zn 253 ± 139 |

lower status of both of the elements. It has been remarked in other studies that Se from the enzyme glutathione peroxidase is a prerequisite for detoxification of a heavy metal (Hirota, 1986; Kazuhide, 1985).

*e. Liver compared to other sampling materials*

Milk samples have been used by our group as a source of information on the selenium status of milk cattle. Milk has small disadvantage in that it does not reflect well inorganic selenium from mineral additions to the feed (Binnerts et al., 1984). Whole blood and isolated red blood cells are excellent sources of information, whether or not the selenium itself is detected, or the Se-enzyme glutathione peroxidase. Thus, for instance, the selenium status of cows (Binnerts, 1979), and that of young animals (Counotte & Hartmans, 1990) have been measured in the Netherlands. The drawback of these materials is the long biological life of the cells, so that the analysis reveals essentially the Se-status of some months earlier. Blood platelets and the serum enzyme have much shorter response (Levander et al., 1983) but are more difficult to harvest and to maintain. Newer insight is that also liver samples can give a good and direct measure of the selenium status adaptable for routine analysis (literature references in Introduction and Discussion, Section a; also McDowell et al., 1990).

*f. Precaution and treatment of selenium deficiency*

The preceding results and discussion reveal that milk producing cows at present are well protected against selenium deficiency. This confirms our results with milk analysis (Binnerts & Viets, 1986). At risk of deficiency are then the animals receiving little or no concentrates. Among them heifers may specially suffer from retained placenta at delivery. Wentink et al., 1988, in a selenium deficient region of the

Netherlands counteracted such a deficiency by including the heifers in the group fed concentrates, already one week before calving. In order to protect other classes of young animals they administered selenium fortified feeds. Provision with extra selenium should be practized even on a wider scale, especially so where and when (imported) concentrates are not or less well available.

## Acknowledgements

The liver material was collected by Ir. H. Klok, (M.Sc. thesis no 339, Department of Human and Animal Physiology, Wageningen Agricultural University). We thank all officials at the slaughterhouses for their kind cooperation. Mr. B.J.T. Nieuwendijk performed the selenium analysis of neutron activation with the FASY system. Ir. Y.T. Bakker and Ir. P. Henkens of the Netherlands Mineral Nutrition Commission have contributed by supplying values on selenium content of feeds.

## References

Behne, D., A. Kyriakopoulos, S. Scheid & H. Gessner, 1991. Effects of chemical form and dosage on the incorporation of selenium into tissue proteins in rats. *Journal of Nutrition* 121: 806-814.

Bisbjerg, B., P. Jochumsen & N.O. Rasbech, 1970. Selenium content in organs, milk and fodder of the cow. *Nordisk Veterinaermedicin* 22: 532-535.

Binnerts, W.T., 1979. The selenium status of dairy cows in the Netherlands as derived from milk and blood analysis. *Netherlands Milk and Dairy Journal* 33: 24-30.

Binnerts, W.T., J. Rijken & T.C. Viets, 1984. The selenium content in milk as an indicator of the selenium status of cows. In: P. Brätter & Schramel (Eds.), Trace Element Analytic Chemistry in Medicine and Biology, Vol. 3, p. 129-137. W. de Gruyter, Berlin-New York.

Binnerts, W.T., 1986. The copper status of cattle in the Netherlands. *Tijdschrift voor Diergeneeskunde* 111: 321-324.

Binnerts, W.T. & T.C. Viets, 1986. Selenium metabolism: liver copper and zinc in cows. In: M. Anke et al. (Eds.) 5, Spurenelementsymposium, Boden, Pflanze, Tier, Mensch Friedrich Schiller Universität, Jena

Binnerts, W.T., 1989. Zinc status of cows as deduced from the liver zinc content. *Netherlands Journal of Agricultural Science* 37: 107-117.

Butler, J.A., P.D. Whanger, A.J. Kaneps & N.M. Patton, 1990. Metabolism of selenite and selenomethionine in the Rhesus monkey, *Journal of Nutrition* 120: 751-759.

Counotte, G.H.M. & J. Hartmans, 1989. Relation between selenium content and glutathionperoxidase activity in blood of cattle. *Veterinary Quarterly* 11: 155-160.

Das, H.A., A. Faanhof & H.A. van der Sloot, 1979. Errors in instrumental neutron activation analysis. *Journal of Radioanalytical Chemistry* 54, 289-301, and 303-310.

Fields, M. et al. 1984. The severity of copper deficiency in rats is determined by the type of dietary carbohydrates. *Proceedings of the Society for Experimental Biology and Medicine* 175: 530-537.

Froeslie, A., J.T. Karlsen & J. Rygge, 1980. Selenium in animal nutrition in Norway. *Acta Agriculturae Scandinavica* 30: 17-25.

Hirota, Y., 1986. Effect of methylmercury on the activity of glutathione peroxidase in rats liver. *Biological Abstracts* 82(11) 107481.

Janghorbani, M., C.S. Mooers, M.A. Smith, H. Terrence, K. Blanock & B.T.G. Ting, 1991. Correlation between the size of the selenite-exchangeable pool and total body or liver selenium in rats. *Journal of Nutrition* 121: 345-354.

Kazuhide, K. et al., 1985. Interaction of methyl mercury and selenium in rats tissue homogenates. *Biological Abstracts* 80(9) 82205.

Korsrud, G. et al., 1985. Trace element levels in liver and kidney from cattle, swine and poultry slaughtered in Canada. *Chemical Abstracts* 103: 69966g

Lane, H.W., R. Strength, J. Johnson & M. White, 1991. Effect of chemical form of selenium on tissue glutathione peroxidase activity in developing rats. *Journal of Nutrition* 121: 80-86.

Levander, O.A., 1986. Selenium. In: W. Mertz (Ed.), Trace Elements in Human and Animal Nutrition, 5th ed., Vol. 2, p. 209-279. Academic Press, Orlando.

Levander, O.A., G. Alfthan, H. Arvillomi, C.G. Gref, J.K. Huttunen, M. Kataja, R. Koivistoinen & J. Pakkarainen, 1983, *American Journal of Clinical Nutrition* 39: 887-888.

McDowell, L.R., 1985. Nutrition of grazing ruminants in warm climates. Academic Press, Orlando.

McDowell, L.R., Y. Salik, J.F. Hentges & C.L. Wilcox, 1990. Selenium supplemen- tation and concentrations of selenium in cattle tissues and fluids. *Nutrition Research (New York)* 10, 793-800.

Nieuwendijk, B.J.T., J.W.R. Woittiez & H.A. Das, 1985. Correction for residual dead-time losses in INAA based on short-lived radionuclides. *Journal of Radioanalysis and Nuclear Chemistry* 89, 465-471.

Norheim, G. et al., 1987. Levels of selenium in human and pig liver: a comparative study. *Chemical Abstracts* 106: 66177b.

Prabowo, A., L.R. McDowell, N,.S. Wilkinson, C.J. Wilcox & J.H. Conrad, 1990. Trace mineral status of grazing cattle in South Sulawesi, Indonesia. In: B. Momcilovic (Ed.), Tema 7: Trace Elements in Man and Animals, no. 7: 27-3. Institute for Medical Research and Occupational Health, University of Zagreb, Croatia, Yugoslavia.

Reiter, R., R. Otter & A. Wendel, 1989. Subcellular distribution of selenium in deficient mouse liver. *Biochemical Journal* J. 258: 541-545.

van Ryssen, J.B.J., J.T. Deagen, M.A. Beilstein & P.D. Whanger, 1989. Comparative metabolism of organic and inorganic selenium by sheep, *Journal of Agricultural and Food Chemistry* 37: 1358-1363.

Schulte, W. & W. Hartfiel, 1987. Selengehalte in Futter- und Nahrungsmitteln. VDLUFA Schriftenreihe 21: 110-111.

Stevenson, L.M., D.G. Jones & N.F. Suttle, 1990. Priority of antibody production for copper and selenium supplies in depleted cattle. In: B. Momcilovic (Ed.), Trace Elements in Man and Animals 7: 27-4. Institute for Medical Research and Occupational Health, IMI, Zagreb, Croatia, Yugoslavia.

Sunde, R.A., 1990. Molecular biology of selenoproteins, *Annual Review of Nutrition* 10: 451-474.

Wentink, G.H., J.A.M. Duivelshof & G.H.M. Counotte, 1988. Selenium deficiency as a cause of retained placentae. *Tijdschrift voor Diergeneeskunde* 113: 624-626.

Woittiez, J.R.W. & B.J.T. Nieuwendijk, 1987. Analysis of selenium in environmental biological samples by neutron activiation. *Journal of Radioanalysis and Nuclear Chemistry: Articles* 110: 603-611.

Woittiez, J.R.W., H.A. Das & B.J.T. Nieuwendijk, 1988. Reactor-based INAA with a fast rabbit system, A summary of 15 years practice. *Journal of Trace and Microprobe Technique* 6, 181-196.