# **Hepatic Cobalt and Copper Levels in Lambs in Norway**

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Sivertsen T, Plassen C: Hepatic cobalt and copper levels in lambs in Norway. Acta vet. scand. 2004, 45, 69-77. – Cobalt and copper concentrations were measured in 599 lamb livers collected at slaughter from 58 sheep flocks in 6 different parts of Norway in 1993. Information about pasture, additional feeding and mineral supplements in the flocks was obtained through a questionnaire. Average hepatic levels of cobalt in the lamb flocks varied from <0.003 to 0.22  $\mu$ g/g ww, and of copper from 5 to 240  $\mu$ g/g ww. Flocks with deficient or marginal cobalt status were found in all parts of southern Norway, but primarily in the west and south-west. Some flocks with marginal copper status were found in the south-west, while flocks with signs of excessive hepatic copper concentrations were found mainly in inner parts of central and northern Norway. Hepatic copper concentrations were significantly higher in lambs that had grazed mountain pastures than in those that had grazed lowland pastures in the summer.

cobalt deficiency; copper poisoning; copper deficiency; trace elements; lambs, pasture; Norway.

#### Introduction

Cobalt deficiency in the form of ovine whiteliver disease (OWLD) is a documented problem in lambs grazing cultivated pastures in the south-western parts of Norway (Ulvund 1990a). Through research and dissemination of information, the Section for Small Ruminant Diseases of the Norwegian School of Veterinary Science has made sheep farmers in this area aware of the problem. Some cases are reported from other Norwegian coastal districts (Ulvund 1995, Ulvund & Pestalozzi 1996). In the inland districts, the disease has almost never been reported. However, as the primary sign of the disease is simply illthrift in grazing lambs, it may be unnoticed and under-diagnosed in areas where it is less known. On this basis we decided to undertake a survey of hepatic cobalt levels in lambs from different parts of Norway.

Copper is another element of concern in sheep

husbandry. Swayback and other distinct clinical symptoms of copper deficiency are uncommon in sheep in Norway (Ulvund 2003), but less obvious signs of production loss or subclinical deficiency may go unrecognised. On the other hand, cases of chronic copper poisoning with acute haemolytic crisis occur regularly in a number of inland districts, and may be a serious problem in some flocks (Sivertsen & Wie 1996). Although copper accumulation serious enough to induce acute toxicity is mainly seen in adult ewes, our experience indicates a strong relationship between sheep and lamb levels on a flock basis. A survey of hepatic copper levels in sheep in Norway was done in 1975 (Frøslie 1977, 1980). Hepatic copper accumulation in sheep in Norway is primarily influenced by molybdenum levels in pasture and roughage (Frøslie & Norheim 1983). Soil acidity and other factors governing the uptake of molybdenum in plants may be influenced by anthropogenic changes in the environment (*Frank et al.* 1994). Hepatic copper concentrations in lambs in Norway might therefore have changed in 2 decades. On this basis, we included analysis of hepatic copper levels in the survey.

The material was collected in the autumn of 1993, at 6 abattoirs in different parts of Norway (Fig 1). As we expected that a considerable number of the lambs in some districts were treated prophylactically with cobalt supplements, we also included a questionnaire to obtain information about pasture and feeding in each of the sampled flocks.

#### Materials and methods

### Collection of samples

To obtain a representative range of samples from different parts of Norway, 6 abattoirs in different counties were chosen: At Forus in Rogaland, Rudshøgda in Hedmark (serving several counties in south-east Norway), Førde in Sogn og Fjordane, Meråker in Nord-Trøndelag, Sortland in Nordland (district of Vesterålen) and Målselv in inner Troms. The abattoirs at Forus, Førde and Sortland receive animals mainly from coastal districts, while those at Rudshøgda, Meråker and Målselv serve typical inland areas (Fig. 1).

The meat inspection veterinary officers at each of these abattoirs were asked to collect 10 lamb liver samples from each of 5 randomly selected flocks from which lambs were delivered to slaughter some day in the beginning of the slaughtering season (September-early October 1993), and from 5 flocks at some day late in the season (late October-November 1993). Within this framework we encouraged the collection of 2 sets of samples from one farm, at each abattoir. This was achieved at 2 abattoirs (Table 2). All the liver samples were collected in plastic bags and frozen before transport to the labora-

tory. Each group of samples was marked with date of slaughter and name or abattoir registration number of the sheep owner. For 3 of the sample sets from Meråker, this marking was destroyed during transport, so the identities of these sheep owners were lost.

# Analytical procedures

Cobalt and copper concentrations in each liver sample were measured by atomic absorption spectroscopy after wet oxidative digestion. The samples were digested in a mixture of concentrated nitric and perchloric acids (Romil Super Purity<sup>1</sup>) in a Tecator Digestive System<sup>2</sup> heating unit. The cobalt analyses were performed with a Varian SpectrAA-3003 with GTA-96 Graphite Tube Atomizer<sup>3</sup>, and the copper analyses by flame atomic absorption with a Varian SpectrAA-6003. Results were calculated and reported on a wet weight basis. Detection limits were 0.003 µg/g for cobalt and 0.5 µg/g for copper. A quality control system including regular analyses of certified reference material (NBS Bovine Liver 1577b<sup>4</sup> and Bovine Liver BCR 185<sup>5</sup>) was adopted. All results of the quality control analyses were within defined limits.

# Questionnaire study

Names and addresses of the 55 identifiable sheep owners were obtained from the abattoirs. A questionnaire was sent to all these farmers asking for the following information: the size of their flock; what kind of pasture the flock had been to (mountain pastures, uncultivated low-land pastures or cultivated pastures); specifica-

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<sup>&</sup>lt;sup>4</sup> National Institute of Standards and Technology, Gaithersburg, USA.

<sup>&</sup>lt;sup>5</sup> Community Bureau of Reference – BCR, Brussel, Belgium.

tion of the periods spent on different kinds of pastures; and what kind of additional feed (if any) the flock had been given in autumn. Finally, the owners were asked if these lambs had received any kind of cobalt supplement or mixed mineral supplement at any time.

Statistical analysis was done with a MS Excel 4.5 program. Differences between groups were analysed by Student's t-test. When studying differences between geographical districts and the effects of pasture history on cobalt and copper levels, the statistical tests were performed both with individual lamb values and with flock means as independent variables. This was done to avoid overestimating the statistical significance of the differences. Unless stated otherwise, the limit of statistical significance was set at p<0.05.

#### Results

# Samples

From one flock we received only 9 samples. Otherwise, a complete set of 10 liver samples was acquired from each of the selected lamb groups. All 599 samples were analysed. One flock slaughtered at Forus and one slaughtered at Førde were sampled twice. The samples thus originated from 58 flocks of sheep, from farms located in 3 coastal and 3 inland districts in Norway (Fig. 1). The first 5 sets of samples from each district (Table 2) were collected between September 1st and October 6th of 1993, the last 5 between October 20th and November 25th of 1993.

#### Analytical results

Means and ranges for all samples collected at each of the 6 different abattoirs are presented in Table 1. Table 2 presents the means and ranges for each set of samples.

# *Questionnaire study*

One of the 55 dispatched questionnaires was re-



Figure 1. Geographical location of the participating abattoirs (crosses) and the farms of the sampled sheep flocks (dots). Numbers indicate geographical areas (location of the abattoir in brackets). 1: Rogaland county (Forus). 2: South-east Norway (Rudshøgda). 3: Sogn og Fjordane county (Førde). 4: Nord-Trøndelag county (Meråker). 5: Vesterålen district (Sortland). 6: Inner part of Troms county (Målselv).

turned because the address could not be found. Of the remaining 54 sheep farmers, 43 answered the questionnaire directly, 5 answered after a reminding letter, and the last 6 were interviewed by telephone. The geographical locations of the farms are shown in Fig. 1.

Seven farmers had used cobalt supplements (Table 2) in the form of cobalt-enriched fertilizer on the pastures (2), cobalt licks (4) and/or orally dosed cobalt pellets (2). All these farms

| District (Abattoir in brackets) | Cobalt µg/g ww |                | Copper μg/g ww   |          |
|---------------------------------|----------------|----------------|------------------|----------|
|                                 | Mean           | Range          | Mean             | Range    |
| Rogaland (Forus)                | 0.031a         | (<0.003-0.082) | 23 <sup>m</sup>  | (4-155)  |
| SE Norway (Rudshøgda)           | $0.032^{a}$    | (<0.003-0.092) | 51 <sup>n</sup>  | (4-175)  |
| Sogn og Fjordane (Førde)        | $0.027^{a}$    | (<0.003-0.081) | 40°              | (5-135)  |
| Nord-Trøndelag* (Meråker)       | $0.047^{b}$    | (<0.003-0.11)  | 124 <sup>p</sup> | (9-600)  |
| Vesterålen (Sortland)           | $0.062^{c}$    | (0.014 - 0.47) | 57 <sup>n</sup>  | (5-230)  |
| Inner Troms (Målselv)           | 0.063°         | (0.014-0.19)   | 119 <sup>p</sup> | (10-350) |
| All samples                     | 0.044          | (<0.003-0.47)  | 69               | (4-600)  |

Table 1. Hepatic cobalt and copper concentrations in 100 lambs slaughtered at each of 6 abattoirs in different regions of Norway.

 $^{a,\,b,\,c\,/\,m,\,n,\,o,\,p}$  : Means with identical letters are not significantly different.

were located in the county of Rogaland. One farmer in the district of Vesterålen had used a special mineral supplement that was unknown to the laboratory, but most probably contained cobalt.

The pasture histories of the flocks are indicated in Table 2, with capital letters indicating the main summer pasture. Flocks with 2 capital letters had alternated between the 2 kinds of pasture. Of the 54 flocks, 29 had grazed on mountain pastures all or most of the summer. Eleven of these had also grazed on uncultivated lowland pastures, usually before or after their period in the mountains. Twenty-one flocks had grazed on uncultivated lowland pastures, but not in the mountains. Forty-eight of the 50 flocks that had grazed on uncultivated mountain or lowland pastures in the summer spent some time on cultivated home pastures at the farm, usually the last weeks before slaughter. Only 4 flocks had been grazing on cultivated pastures only.

The pasture histories of the flocks were not evenly distributed. In the 3 northern districts, the majority of flocks had grazed in the mountains. In Sogn og Fjordane and in south-east Norway, about half of the flocks had grazed in the mountains, while none of the flocks from

Rogaland had grazed in mountain pastures. The 4 flocks that had grazed only on cultivated pastures were all located in Rogaland (Table2).

# Geographical differences

When calculated on the basis of individual samples, cobalt concentrations in samples from the 2 districts in northern Norway were significantly higher than from other districts, those from the 3 southern districts were significantly lower, while the cobalt concentrations in Nord-Trøndelag (Meråker) were intermediate (Table 1). When calculated with flock means as an independent variable, the average hepatic cobalt concentrations in lambs from inner Troms (Målselv) were still significantly different from those from the 3 southern districts, but other differences between the districts were not significant.

The regional differences in hepatic copper concentrations were larger and more distinct. Samples from the 2 inland districts of Nord-Trøndelag and inner Troms showed high copper concentrations, those from Vesterålen (Sortland) and south-east Norway (Rudshøgda) were intermediate, those from Sogn og Fjordane (Førde) somewhat lower, while samples from Rogaland (Forus) had quite low copper levels.

<sup>\*</sup>n=99.

Table 2. Hepatic cobalt and copper concentrations in lambs from 58 different flocks in Norway, with information about summer pasture for each flock.

| Mean 31 39 27 27 27 27 27 27 28 28 28 28 28 28 28 28 28 28 28 28 28   |              |      |          |                     |             |       |                 |      |           |         |   |
|---|--------------|------|----------|---------------------|-------------|-------|-----------------|------|-----------|---------|---|
| 0.036* (0.019-0.059) 31<br>0.051** (0.014-0.082) 27<br>0.037*a (0.020-0.059) 27<br>0.0356* (0.029-0.047) 20<br>0.011 (<0.003-0.021) 39<br>0.042** (0.031-0.064) 21<br>0.033*a (0.027-0.041) 14<br>0.066*! (<0.003-0.009) 14<br>0.034 (0.004-0.057) 5<br>0.037 (0.011-0.062) 27<br>0.037 (0.011-0.062) 27<br>0.045 (0.013-0.045) 22<br>0.045 (0.013-0.045) 28<br>0.025b (0.013-0.045) 28<br>0.025b (0.002-0.051) 16<br>0.025b (0.002-0.052) 28<br>0.042 (0.028-0.052) 28<br>0.042 (0.028-0.053) 37<br>0.042 (0.028-0.053) 37<br>0.038 (0.023-0.043) 59<br>0.017 (0.003-0.043) 59<br>0.017 (0.014-0.043) 59<br>0.035 (0.014-0.047) 91<br>0.031 (0.021-0.041) 29 |              | Mean | Range    | pasture*** District | District    | Mean  | Range           | Mean | Range     | pasture |   |
| 0.051** (0.014-0.082) 27<br>0.037*a (0.020-0.059) 27<br>0.036** (0.020-0.059) 27<br>0.042** (0.031-0.064) 21<br>0.033*a (0.027-0.041) 14<br>0.006*! (<0.003-0.009) 14<br>0.034 (0.004-0.057) 5<br>0.037 (0.011-0.062) 27<br>0.042 (0.013-0.045) 51<br>0.045 (0.013-0.045) 22<br>0.045 (0.013-0.045) 16<br>0.045 (0.033-0.066) 34<br>0.045 (0.033-0.066) 34<br>0.045 (0.033-0.065) 88<br>0.045 (0.003-0.055) 68<br>0.045 (0.003-0.055) 68<br>0.045 (0.003-0.043) 16<br>0.038 (0.023-0.059) 18<br><0.003 All<0.003<br>0.017 <0.003-0.003<br>0.017 <0.003-0.003<br>0.017 (0.014-0.043) 59<br>0.031 (0.021-0.041) 29<br>0.077 (0.014-0.071) 29                    | _            |      | (5-63)   | C                   | South-east  | 0.034 | (<0.003-0.051)  | 50   | (14-115)  | L, c    | l |
| 0.037*a (0.020-0.059) 27<br>0.036* (0.029-0.047) 20<br>0.011 (<0.003-0.021) 39<br>0.042* (0.031-0.064) 21<br>0.033*a (0.027-0.041) 14<br>0.006*! (<0.003-0.009) 14<br>0.034 (0.004-0.057) 5<br>0.030* (0.011-0.062) 27<br>0.045 (0.017-0.055) 51<br>0.025b (0.013-0.045) 22<br>0.045 (0.033-0.066) 34<br>0.025b (0.013-0.045) 22<br>0.045 (0.033-0.060) 18<br>0.025b (0.003-0.043) 16<br>0.038 (0.023-0.043) 16<br>0.038 (0.023-0.043) 16<br>0.038 (0.023-0.043) 59<br>0.017 <0.003 All<0.003<br>0.017 <0.003-0.043) 59<br>0.035 (0.014-0.043) 59<br>0.031 (0.021-0.041) 29<br>0.077 (0.014-0.047) 29   |              |      | (16-48)  | C                   | Norway      | 0.012 | (<0.003-0.031)  | 48   | (22-100)  | М, с    |   |
| 0.036* (0.029-0.047) 20<br>0.011 (<0.039-0.021) 39<br>0.042* (0.031-0.064) 21<br>0.033*a (0.021-0.064) 21<br>0.033*a (0.021-0.064) 14<br>0.034 (0.004-0.057) 5<br>0.037 (0.011-0.062) 27<br>0.045 (0.013-0.045) 22<br>0.045 (0.013-0.045) 21<br>0.045 (0.013-0.045) 18<br>0.025b (0.013-0.045) 22<br>0.045 (0.035-0.052) 18<br>0.035 (0.028-0.053) 18<br>0.038 (0.023-0.043) 16<br>0.038 (0.023-0.043) 16<br>0.034 (0.023-0.043) 59<br>0.017 <0.003-0.043) 59<br>0.035 (0.014-0.047) 91<br>0.035 (0.014-0.047) 36<br>0.027 (0.014-0.047) 29<br>0.027 (0.014-0.047) 29   | ٠<br>۳       |      | (4-155)  | C                   | (Rudshøgda) | 0.041 | (0.009-0.073)   | 27   | (11-86)   | L, c    |   |
| 0.011 (<0.003-0.021) 39<br>0.042** (0.031-0.064) 21<br>0.033*a (0.027-0.041) 14<br>0.034* (0.004-0.057) 5<br>0.034* (0.004-0.057) 5<br>0.037* (0.011-0.062) 27<br>0.025b (0.013-0.045) 22<br>0.025b (0.013-0.045) 22<br>0.045 (0.013-0.045) 22<br>0.025b (0.013-0.045) 18<br>0.025b (0.005-0.045) 16<br>0.038 (0.023-0.059) 18<br><0.003 All <0.003<br>0.017 <0.003-0.031 66<br>0.017 <0.003-0.043 59<br>0.034 (0.023-0.043) 59<br>0.035 (0.014-0.047) 91<br>0.035 (0.014-0.047) 36<br>0.027 (0.014-0.047) 29<br>0.027 (0.014-0.041) 29   | _            |      | (4-32)   | L, c                |             | 0.063 | (0.036-0.092)   | 33   | (18-60)   | Г, с    |   |
| 0.042* (0.031-0.064) 21<br>0.033*a (0.027-0.041) 14<br>0.006*! (<0.003-0.009) 14<br>0.034 (0.004-0.057) 5<br>0.030* (0.011-0.062) 27<br>0.035 (0.011-0.065) 51<br>0.025b (0.013-0.045) 22<br>0.045 (0.013-0.045) 22<br>0.045 (0.035-0.052) 28<br>0.045 (0.035-0.052) 28<br>0.045 (0.003-0.043) 16<br>0.038 (0.032-0.043) 16<br>0.038 (0.030-0.03) 37<br><0.003 All <0.003<br>0.017 <0.003-0.043) 59<br>0.017 (0.014-0.047) 91<br>0.031 (0.021-0.041) 36<br>0.027 (0.014-0.057) 29   | _            | _    | (11-61)  | Γ                   |             | 0.022 | (0.015-0.032)   | 37   | (4-70)    | L, c    |   |
| 0.033*a (0.027-0.041) 14<br>0.006*! (-0.003-0.009) 14<br>0.034 (0.004-0.057) 5<br>0.037 (0.011-0.062) 27<br>0.025b (0.013-0.066) 34<br>0.042 (0.013-0.045) 22<br>0.042 (0.035-0.052) 28<br>0.042 (0.035-0.052) 28<br>0.042 (0.035-0.052) 28<br>0.042 (0.035-0.053) 68<br>0.035b (0.003-0.043) 16<br>0.038 (0.023-0.043) 16<br>0.038 (0.023-0.043) 59<br>0.017 (-0.003 All <-0.003 66<br>0.017 (-0.003 O.003-0.081) 58<br>0.035 (0.014-0.047) 91<br>0.031 (0.021-0.041) 29<br>0.077 (0.014-0.057) 29   | _            |      | (15-31)  | C                   |             | 0.037 | (0.024-0.053)   | 19   | (11-29)   | L, c    |   |
| 0.006*! (<0.003-0.009) 14<br>0.034 (0.004-0.057) 5<br>0.030* (0.011-0.062) 27<br>0.037 (0.017-0.055) 51<br>0.025b (0.013-0.045) 22<br>0.042 (0.035-0.052) 68<br>0.042 (0.025-0.052) 68<br>0.042 (0.028-0.053) 68<br>0.035b (0.028-0.053) 68<br>0.038 (0.023-0.043) 16<br>0.038 (0.023-0.043) 16<br>0.017 <0.003 All<0.003 66<br>0.017 <0.003-0.003 66<br>0.017 (0.014-0.043) 59<br>0.035 (0.014-0.041) 35<br>0.037 (0.014-0.041) 29   | _            | _    | (5-42)   | C                   |             | 0.026 | (0.014-0.033)   | 99   | (34-105)  | M, 1, c |   |
| 0.034 (0.004-0.057) 5<br>0.030* (0.011-0.062) 27<br>0.045 (0.017-0.055) 34<br>0.025b (0.013-0.045) 22<br>0.045 (0.013-0.045) 22<br>0.045 (0.038-0.055) 68<br>0.025b (0.008-0.055) 68<br>0.025b (0.006-0.043) 16<br>0.038 (0.0023-0.043) 16<br>0.038 (0.003-0.043) 16<br>0.017 <0.003 All<0.003<br>0.017 <0.003 -0.003<br>0.017 (0.014-0.041) 59<br>0.031 (0.011-0.041) 36<br>0.077 (0.014-0.041) 29   | Ĭ            | _    | (5-42)   | L, c                |             | 0.021 | (0.006-0.035)   | 73   | (21-115)  | М, с    |   |
| 0.030* (0.011-0.062) 27 0.045 (0.033-0.066) 34 0.037 (0.017-0.055) 51 0.025b (0.013-0.045) 22 0.042 (0.035-0.052) 28 0.025b (0.008-0.043) 16 0.038 (0.023-0.053) 18 <0.003 All <0.003 0.017 <0.003-0.003 50 0.017 <0.003-0.043 59 0.034 (0.023-0.043) 59 0.035 (0.014-0.047) 91 0.035 (0.014-0.047) 36 0.027 (0.014-0.041) 29   | _            | _    | (4-6)    | L, C                |             | 0.026 | (0.003-0.064)   | 105  | (70-175)  | М, с    |   |
| 0.045 (0.033-0.066) 34<br>0.037 (0.017-0.055) 51<br>0.025b (0.013-0.045) 22<br>0.045 (0.035-0.052) 28<br>0.025b (0.006-0.043) 16<br>0.038 (0.0023-0.050) 18<br><0.003 All<0.003 18<br><0.003 All<0.003 57<br><0.003 (0.003-0.081) 58<br>0.017 <0.003-0.043) 59<br>0.017 (0.014-0.041) 91<br>0.031 (0.021-0.041) 29<br>0.077 (0.014-0.041) 29  |              | _    | (10-45)  | L, c                |             | 0.040 | (0.023-0.058)   | 48   | (11-105)  | L, c    |   |
| 0.037 (0.017-0.055) 51<br>0.025b (0.013-0.045) 22<br>0.042 (0.028-0.052) 28<br>0.025b (0.006-0.043) 16<br>0.038 (0.023-0.050) 37<br><0.003 All<0.003 37<br><0.003 All<0.003 66<br>0.017 <0.003-0.081 58<br>0.017 <0.003-0.081 58<br>0.017 (0.014-0.047) 91<br>0.035 (0.014-0.047) 91<br>0.037 (0.014-0.041) 29<br>0.071 (0.016-0.11) 29   |              | _    | (18-58)  | L, c                | Nord-       | 0.032 | (0.021-0.039)   | 74   | (38-145)  | M, L, c |   |
| 0.025b (0.013-0.045) 22<br>0.045 (0.035-0.052) 28<br>0.025b (0.006-0.043) 16<br>0.038 (0.023-0.050) 18<br><0.003 All<0.003 66<br>0.017 <0.003 66<br>0.017 <0.003 67<br>0.034 (0.023-0.043) 59<br>0.035 (0.014-0.041) 36<br>0.077 (0.014-0.041) 29<br>0.071 (0.016-0.11) 29  |              | 51   | (7-110)  | М, с                | Trøndelag   | 0.037 | (0.024-0.058)   | 09   | (30-96)   | M, l, c |   |
| 0.045 (0.035-0.052) 28<br>0.042 (0.028-0.055) 68<br>0.025b (0.006-0.043) 16<br>0.038 (0.023-0.050) 18<br><0.003 All<0.003 37<br><0.003 All<0.003 66<br>0.017 <0.003-0.081 58<br>0.034 (0.023-0.043) 59<br>0.035 (0.014-0.047) 91<br>0.037 (0.014-0.047) 29<br>0.077 (0.014-0.057) 29  |              | _    | (7-35)   | Γ                   | (Meråker)   | 0.054 | (0.045-0.079)   | 64   | (25-125)  | М, с    |   |
| 0.042 (0.028-0.055) 68<br>0.025b (0.006-0.043) 16<br>0.038 (0.023-0.050) 18<br><0.003 All<0.003 37<br><0.003 All<0.003 66<br>0.017 <0.003-0.081 58<br>10 0.034 (0.023-0.043) 59<br>0.035 (0.014-0.047) 91<br>0.031 (0.021-0.041) 36<br>0.027 (0.014-0.057) 29<br>0.071 (0.016-0.11) 29  |              | _    | (11-61)  | $\mathbb{Z}$        |             | 0.038 | (0.029-0.054)   | 155  | (85-230)  | M, L, c |   |
| 0.025b (0.006-0.043) 16<br>0.038 (0.023-0.050) 18<br><0.003 AII<0.003 37<br><0.003 AII<0.003 66<br>0.017 <0.003-0.081 58<br>10 0.034 (0.023-0.043) 59<br>0.035 (0.014-0.047) 91<br>0.031 (0.021-0.041) 36<br>0.027 (0.014-0.057) 29<br>0.071 (0.016-0.11) 29  |              | _    | (23-135) | M, 1, c             |             | 0.030 | (0.020-0.047)   | 46   | (9-165)   | M, 1, c |   |
| 0.038 (0.023-0.050) 18<br><0.003 All <0.003 37<br><0.003 All <0.003 66<br>0.017 <0.003-0.081 58<br>1 0.034 (0.023-0.043) 59<br>0.035 (0.014-0.047) 91<br>0.031 (0.021-0.041) 36<br>0.027 (0.014-0.057) 29<br>0.071 (0.016-0.11) 29  | _            | _    | (5-42)   | L, c                |             | 0.037 | (0.021-0.055)   | 160  | (86-260)  | ı       |   |
| <ul> <li>&lt;0.003 All &lt;0.003 37</li> <li>&lt;0.003 All &lt;0.003 66</li> <li>&lt;0.017 &lt;0.003-0.081 58</li> <li>&lt;0.034 (0.023-0.043) 59</li> <li>&lt;0.035 (0.014-0.047) 91</li> <li>&lt;0.031 (0.021-0.041) 36</li> <li>&lt;0.027 (0.014-0.057) 29</li> <li>&lt;0.071 (0.016-0.11) 29</li> </ul>   |              | _    | (9-32)   | Г, с                |             | 0.015 | (<0.003-0.031)  | 130  | (100-160) | М, с    |   |
| <ul> <li>&lt;0.003 All &lt;0.003 66</li> <li>0.017 &lt;0.003-0.081 58</li> <li>0.034 (0.023-0.043) 59</li> <li>0.035 (0.014-0.047) 91</li> <li>0.027 (0.014-0.041) 36</li> <li>0.027 (0.014-0.057) 29</li> <li>0.071 (0.016-0.11) 29</li> </ul>   | ·            | 37   | (09-6)   | L, c                |             | 0.085 | (0.028-0.105)   | 200  | (74-430)  |         |   |
| 0.017 <0.003-0.081 58<br>0.034 (0.023-0.043) 59<br>0.035 (0.014-0.047) 91<br>0.027 (0.014-0.057) 29<br>0.071 (0.016-0.11) 29  | •            | 99   | (38-92)  | М, с                |             | 0.076 | (0.065-0.089)   | 115  | (49-195)  | L, c    |   |
| 0.034 (0.023-0.043) 59<br>0.035 (0.014-0.047) 91<br>0.031 (0.021-0.041) 36<br>0.027 (0.014-0.057) 29<br>0.071 (0.016-0.11) 29   | ·            | 28   | (19-115) | М, с                |             | 0.059 | (0.051 - 0.068) | 240  | (130-600) | ,       |   |
| 0.035 (0.014-0.047) 91<br>0.031 (0.021-0.041) 36<br>0.027 (0.014-0.057) 29<br>0.071 (0.016-0.11) 29   | (0.023-0.043 | _    | (8-110)  | M, c                | Inner Troms | 0.037 | (0.015-0.052)   | 130  | (89-190)  | M, 1, c |   |
| (0.021-0.041) 36<br>(0.014-0.057) 29<br>(0.016-0.11) 29   | (0.014-0.047 | _    | (16-150) | М, с                | (Målselv)   | 0.048 | (0.034-0.069)   | 105  | (23-170)  | M, 1, c |   |
| $ \begin{array}{ccc} (0.014 \text{-} 0.057) & 29 \\ (0.016 \text{-} 0.11) & 29 \end{array} $  | (0.021-0.041 | _    | (5-175)  | М, с                |             | 0.070 | (0.057 - 0.094) | 105  | (54-160)  | L, c    |   |
| (0.016-0.11) 29   | (0.014-0.057 | ) 29 | (98-6)   | М, с                |             | 0.14  | (0.093-0.19)    | 135  | (93-220)  | M, 1, c |   |
|   | (0.016-0.11) |      | (9-74)   | L, c                |             | 0.072 | (0.025-0.17)    | 120  | (57-175)  | L, c    |   |
| (0.043-0.062) 25  | (0.043-0.062 | _    | (8-63)   | L, c                |             | 0.057 | (0.045-0.071)   | 130  | (82-250)  | М, с    |   |
| (0.019-0.041) 46  | (0.019-0.041 | •    | (25-66)  | М, с                |             | 0.042 | (0.030-0.055)   | 95   | (55-190)  | M, L, c |   |
| (0.025-0.042) 110   | (0.025-0.042 | _    | (35-170) | М, с                |             | 0.049 | (0.014-0.083)   | 160  | (81-350)  | M, l, c |   |
| (0.041-0.16) 51   | (0.041-0.16) | 51   | (13-125) | L, c                |             | 0.056 | (0.038-0.083)   | 80   | (10-135)  | М, с    |   |
| _   | (0.048-0.47) | 100  | (59-230) | М, с                |             | 0.056 | (0.038-0.068)   | 130  | (84-195)  |         |   |

\* Used cobalt supplementation (according to answers in questionnaire) \*\*Used unknown mineral supplement probably containing cobalt a-a; b-b: Sample sets from the same flock of sheep. ! Unlikely result. (See discussion).
\*\*\*Summer pasture. M: Mountain pasture. L/I: Uncultivated lowland pasture C/ c: Cultivated lowland pasture. -: No information. Capital letters indicate the main summer pasture (see text)

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All these differences were statistically significant when calculated on individual sample basis (Table 1). When calculated on the basis of flock means, the differences remained statistically significant, with the exception of the difference between samples from south-east Norway and Sogn og Fjordane.

Effect of summer pasture and time of slaughter In lambs from south-east Norway, hepatic cobalt concentrations were somewhat higher in lambs that had grazed the main part of the summer on lowland pastures than in those that had grazed in the mountains. This difference was significant by calculation on individual values, but not when calculated on flock means. In other districts, or in the entire set of samples, there were no significant differences in cobalt levels between the flocks that had grazed in the mountains and those that had grazed on lowland pastures, or between uncultivated and cultivated lowland pastures.

In the entire set of samples, the mean hepatic copper concentration in lambs that had grazed in the mountains was twice as high as in those that had grazed on uncultivated lowland pastures in the main part of the summer. This difference was highly significant (p<0.001), both on individual sample and flock mean basis. Calculated district by district, the difference was significant or nearly significant in south-east  $\begin{aligned} & \text{Norway } (p_{\text{individual}} \!\!<\!\! 0.001, \, p_{\text{flock}} \!\!<\!\! 0.05), \, \text{Sogn og} \\ & \text{Fjordane} \quad (p_{\text{individual}} \!\!<\!\! 0.001, \quad p_{\text{flock}} \!\!<\!\! 0.02) \quad \text{and} \end{aligned}$ Vesterålen ( $p_{individual}$ <0.001,  $p_{flock} \sim 0.06$ ), but not in inner Troms. In Nord-Trøndelag only one flock had grazed mainly on lowland pastures. There was no difference in the average hepatic cobalt concentrations between lambs slaughtered early and late in the autumn. The mean hepatic copper concentration was somewhat higher in the lambs slaughtered late in autumn than in those slaughtered earlier. This difference was significant when calculated on individual sample basis, but not on flock mean basis. The differences in cobalt concentrations between the geographical districts were most pronounced in the lambs slaughtered late in the season. The geographical differences in copper concentrations were similar in both lamb groups.

In the 2 flocks that were sampled twice, copper concentrations were slightly lower in the lambs that were slaughtered late in the season, while cobalt concentrations were almost identical in the 2 sample sets from each flock (Table 2).

#### Discussion

Normal hepatic cobalt levels in lambs are reported to be between 0.03 and 0.1 µg/g ww (Robertson 1971). According to Radostits et al. (1994), levels below 0.02 µg/g ww (0.07 µg DM) are associated with clinical cobalt deficiency, and  $0.015 \mu g/g$  ww  $(0.05 \mu g DM)$  is considered as a critical level. *Ulvund* (1990b) found mean hepatic cobalt concentrations from 0.013 to 0.024 µg/g ww in groups of lambs with manifest OWLD. We therefore think that an average cobalt level below 0.025 µg/g ww in a sheep flock should be considered marginal. In the present survey we found 9 flocks with average hepatic cobalt levels below 0.025 µg/g ww (Table 2). Five of these were from Rogaland (2) and Sogn og Fjordane (3), and among them 3 flocks were extremely deficient, with hepatic cobalt below 0.010 µg/g ww in all lambs. Three flocks with marginal cobalt levels were found in south-east Norway, and one in Nord-Trøndelag. Individual lambs with hepatic cobalt concentrations below 0.010 µg/g ww were found in 7 flocks in Rogaland and Sogn og Fjordane, 5 flocks in south-east Norway, and one in Nord-Trøndelag. The results confirm that cobalt levels that may induce deficiency are primarily found in the south-western and western parts of Norway, but some flocks may be at risk also in the south-east inland. Recently, Strøm et al.

(2003) observed marginal serum levels of vitamin B12 in sheep from a farm in south-east Norway, indicating a marginal or deficient cobalt status. In northern Norway we found no flocks with marginal hepatic cobalt levels, but cases of deficiency have been reported in coastal areas (*Ulvund* 1995).

Seven out of 9 farmers from Rogaland reported using cobalt supplements (Table 2). This suggests that cobalt supplementation has become widespread in sheep farms in this county. Only one farmer from another area seems to have used a cobalt-containing supplement. This may indicate that cobalt supplementation was still not much used outside the south-west at the time these data were collected. Cobalt supplements have been shown to be very effective in normalizing hepatic cobalt levels in sheep (Ulvund 1990b). In one of the flocks that, according to the questionnaire information, had been given cobalt supplements, the liver samples apparently still indicated typical deficiency levels. This result is so unlikely that we suspect a mistake, e.g. a mix-up of 2 sample sets. All the other flocks that were reported to have been given supplements had adequate mean hepatic cobalt levels. How many of these flocks that would have been deficient without supplements is unknown.

All the 4 flocks in our survey that had grazed on cultivated pastures only were located in Rogaland. Clinically manifest OWLD has mainly been seen in Norway in sheep grazing on cultivated pastures. Our results do not indicate that the type of pasture influences the cobalt levels in the regions studied. This finding supports the conclusion that clinical OWLD on cultivated lowland pastures is connected to high levels of soluble carbohydrates in the grass, giving a heavy load of propionic acid to the liver of the lambs (*Ulvund* 1990a, *Ulvund & Pestalozzi* 1996).

The scarcity of reports on clinical cobalt defi-

ciency outside the western coastal districts may be related to pasturing practices, but it may also indicate a lack of awareness of the problem in other parts of Norway. In our opinion the results of this survey indicate that veterinarians and farmers in all of southern Norway should consider the possibility of cobalt deficiency whenever lambs fail to thrive, especially on cultivated or rich lowland pastures.

Our results confirm the strong regional variation in copper levels observed by Frøslie (1977). Normal hepatic copper levels in sheep are reported to be around 50 µg/g ww, and hepatic concentrations above 150 µg/g ww indicate a risk of chronic copper poisoning (Frøslie 1980, Søli 1980). In our experience, flocks with serious copper toxicity problems tend to have hepatic copper levels considerably above this limit in adult sheep at slaughter in the autumn. Average values above 300 µg/g ww have been observed (Sivertsen & Wie 1996). However, in Norwegian sheep flocks with high hepatic copper concentrations, the levels found in lambs at slaughter are considerably lower than in older sheep. In 2 studies, hepatic copper concentrations in lambs have been found to be 30-60% of concentrations in comparable groups of adult ewes (Frøslie 1977, Sivertsen & Wie 1996). Average hepatic copper concentrations above 150 ug/g ww in lambs do therefore in our opinion indicate a serious copper accumulation problem in the flock. Even levels above 100 µg/g ww in lambs may indicate some risk of copper toxicity for adult sheep from the same farm. In the present survey, we found 15 flocks with

In the present survey, we found 15 flocks with mean hepatic copper concentrations above 100  $\mu$ g/g in the lambs: one in south-east Norway, six in Trøndelag and eight in Troms. Five flocks had average levels above 150  $\mu$ g/g, and 4 of these were from Trøndelag (Table 2). These results confirm that an excess in hepatic copper in sheep in autumn is common in the inland districts of Troms and Trøndelag. This is in line

with the results of *Frøslie* (1977, 1980). On the other hand, hepatic copper levels in the lambs from the south east inland were surprisingly normal. *Frøslie* (1977, 1980) found high hepatic copper levels in sheep from the south east inland districts, similar to the levels found in Trøndelag and northern Norway. The present study includes 60 lambs from 10 flocks in south-east Norway. Obviously, this is a small subset of the lamb population in the area, and local geographical variation may be considerable. Still, the possibility of a real reduction of the copper accumulation problems in southeast Norway cannot be excluded.

Hepatic copper levels below 3-6  $\mu$ g/g ww are reported to indicate deficiency (Frøslie 1980, Radostits et al. 1994). As with cobalt, several unknown factors seem to influence the risk of manifest clinical copper deficiency. In this survey, one flock in Rogaland had a mean hepatic copper level of 5  $\mu$ g/g ww. This indicates that subclinical copper deficiency may be of some importance in the area. However, at the Section for Small Ruminant Diseases in Rogaland, typical swayback or other distinct pathological conditions related to copper deficiency are rarely diagnosed (Ulvund 2003).

In the present survey, copper levels were significantly higher in flocks that had spent the main part of the summer on mountain pastures than in those that had stayed on lowland pastures. In our opinion this is an interesting observation. Studies in a heavily copper loaded sheep flock in northern Nord-Trøndelag indicate that hepatic copper build-up mainly occurred at mountain pasture, even though the harvested roughage fed to this flock in winter did have a very high Cu/Mo ratio (Sivertsen & Wie 1996). The results of the present survey indicate a stronger copper accumulation on mountain pastures than on cultivated and uncultivated lowland pastures, as a general trend in large parts of Norway. The background for this effect may be

an even more skewed Cu/Mo ratio in some important mountain pasture plants (*Garmo et al.* 1986) than in lowland pastures and harvested grass (*Frøslie & Norheim* 1983). This effect of mountain pasture in the summer on hepatic copper accumulation in sheep may limit the opportunities for effective prophylaxis against chronic copper poisoning in Norway.

The time of slaughter had no influence on the hepatic cobalt levels. The influence on copper levels were somewhat contradictory. In the 2 flocks that were sampled twice, hepatic copper concentrations did not change substantially from early to late in the slaughtering season. However, the average copper level in all samples was somewhat higher in lambs slaughtered late in the season. This difference was not significant when calculated on a flock mean basis, and from other experience we suspect that it may be a fortuitous result. In a biopsy study of seasonal variation in hepatic copper concentrations in individual sheep in a Norwegian sheep herd with high copper levels (Sivertsen & Wie 1996), hepatic copper levels changed very little during the autumn months.

# Aknowledgements

We thank the veterinary officers Ola Austvoll, Ari Ek, Marit Forbord, Torill Mørk, Harald Ribe jr., Jacob Skogan and Per Inge Wetteland for their help with collection of the liver samples.

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#### Sammendrag

Kobolt- og kobbernivåer i lever hos norske lam.

Konsentrasjonene av kobolt og kobber i lever ved slakting er undersøkt hos 599 lam i 58 besetninger fra 6 ulike områder i Norge. Ved hjelp av et spørreskjema ble det samlet opplysninger fra hver av besetningene om beite, tilleggsforing og mineraltilskudd lammene hadde fått. De gjennomsnittlige leververdiene av kobolt i lam fra de enkelte besetningene varierte fra <0,003 til 0,22 μg/g våtvekt, og av kobber fra 5 til 240 μg/g. Besetninger med mangelfull eller marginal koboltstatus hos lam forekom i alle deler av Sør-Norge, men særlig i Rogaland og Sogn og Fjordane. Lam fra noen besetninger i Rogaland hadde marginal kobberstatus, mens besetninger med tegn på uheldig kobberopphopning i lever hovedsakelig ble sett i indre Trøndelag og indre Troms. Kobbernivåene i lever var signifikant høyere hos lam som hadde vært på fjellbeite om sommeren enn hos lam som hadde vært på utmark eller kulturbeite i lavlandet.

(Received 1 December 2003; accepted 15 December 2003).

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