LIVER CONCENTRATIONS OF COPPER, COBALT, AND SELENIUM IN WILD NORWEGIAN RED DEER (CERVUS ELAPHUS)

Authors: Vikøren, Turid, Bernhoft, Aksel, Waaler, Thor, and Handeland, Kjell

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Turid Vikøren,[1,4] **Aksel Bernhoft,**[2] **Thor Waaler,**[3] and **Kjell Handeland**[1]

[1] Section of Wildlife Diseases, National Veterinary Institute, PO Box 8156 Dep., NO-0033 Oslo, Norway

[2] Section of Toxicology, National Veterinary Institute, PO Box 8156 Dep., NO-0033 Oslo, Norway

[3] Section of Chemistry, National Veterinary Institute, PO Box 8156 Dep., NO-0033 Oslo, Norway

[4] Corresponding author (email: turid.vikoren@vetinst.no)

ABSTRACT: Liver samples from 245 wild red deer (*Cervus elaphus*) collected during the licensed hunting season in 2001 from five different locations in western Norway were analyzed for copper (Cu), cobalt (Co), and selenium (Se). The associations between these trace elements and geographical location, age group, and sex were studied. The median (and range of) liver concentrations (µg/g wet weight) for all the examined deer were: Cu 20 (1.7–103), Co 0.08 (<0.01–0.18), and Se 0.09 (0.04–1.0). The results indicate a generally low status of Cu and Se. In total, 15 (6%) red deer had deficient Cu levels (<4 µg/g). For all three elements, the liver concentrations showed a significant geographic variation. The geographic difference was most distinct for Cu. The lowest median Cu concentration was found in deer from the island Hitra, where 13% of the animals had deficient Cu levels. Significant differences between age groups were found for all elements, and generally, the adults (≥2.5 yr) had the highest levels. No significant sex differences within the various age groups were found, with three exceptions: female calves and adults had significantly higher Co levels than male deer, and adult males had significantly higher Se levels than adult females. The Cu and Se status of wild red deer in parts of Norway is low; however, the significance of this needs to be explored further.

Key words: *Cervus elaphus,* cobalt, copper, deficiency, red deer, selenium, trace elements.

INTRODUCTION

Among the important trace elements in grazing livestock are copper (Cu), cobalt (Co), and selenium (Se), but little is known about trace element requirements, metabolism, and deficiencies in deer species (Wilson and Grace, 2001). Copper, and to some extent Se, are the trace elements known to cause deficiency problems in farmed red deer (*Cervus elaphus*) (Wilson and Grace, 2001). In Norway, Cu deficiency is a considerable problem in red deer herds, and low Cu status has been associated with enzootic ataxia (Handeland and Flåøyen, 2000), osteochondrosis (Handeland and Bernhoft, 2004), and a disease syndrome characterized by poor growth, emaciation, diarrhea, and reproductive disturbances (K. Handeland, unpubl. data). Also, low liver Se concentration has been found in Norwegian deer herds (Handeland and Bernhoft, 2004). Selenium and vitamin E deficiency in deer has been associated with Se-responsive unthriftiness (Knox et al., 1987; Jones, 1994), postcapture myopathy (van Reenen, 1981),

and white muscle disease (Wilson and Grace, 2001). There have been no confirmed reports of clinical Co deficiency in deer, but low Co levels occasionally have been found in individual farmed red deer in Norway (Handeland and Bernhoft, 2004; K. Handeland, unpubl. data). Marginal to deficient liver Co levels have been registered in grazing lambs in western Norway, some of them suffering from white liver disease (Ulvund, 1990).

In Norway, red deer farming is not yet a large industry; however, the number of herds is rising, with current reckoning standing at 50 farms (Anonymous, 2003). These herds have their origin exclusively in the native wild red deer population; importation of deer has not been practiced because of strict import regulations. Over the past few decades, there has been enormous growth within the wild red deer population in Norway, and the number of red deer shot during each hunting season has increased from 6,453 in 1978 to 24,533 in 2002 (Statistics Norway, 2003). Knowledge of the trace element status in wild red deer

is important with regard to the viability and health of the wild populations and of comparative interest, highlighting changes taking place after capture and during farming. From the farmers' point of view, it is important to have this insight when capturing wild deer for farming purposes so that they are aware of the possible need for nutritional supplementation.

Previous trace element surveys of wild red deer in Norway have shown large individual variation in liver Cu and Se levels (Frøslie et al., 1984; Kålås and Myklebust, 1994; Kålås and Øyan, 1997; Rosef et al., 2001). In general, these studies were small and geographically limited, and data on Co levels were not obtained. Thus, a more comprehensive survey was undertaken as part of the National Health Surveillance Program for Cervids (HOP). The objectives of this study were to 1) examine the liver concentrations of Cu, Co, and Se in wild Norwegian red deer and 2) study the association between these concentrations and geographic location, age, and sex.

MATERIALS AND METHODS

The Norwegian red deer population and study areas

In Norway, the majority of the wild red deer population is found along the west coast (Langvatn et al., 1996). Red deer from five different municipalities in this region; Namsos ($65°30'N$, $11°30'E$), Hitra ($63°35'N$, $8°45'E$), Hareid ($62°20'N$, $6°0'E$), Eid ($61°55'N$, $6°0'E$), and Gaular ($61°20'N$, $5°50'E$) were sampled (Fig. 1). According to Langvatn et al. (1996), Namsos and Hareid are defined as coastal zones, whereas Eid and Gaular are inland. Hitra is an island that is regarded as a separate region because of the extreme maritime climate and a sedentary red deer population. The population density index of red deer in a given municipality may be shown as the total number of animals harvested during the yearly hunting season per 10 km^2 qualifying area (the area of suitable red deer habitat within each municipality; Mysterud et al., 2001; Statistics Norway, 2002). Accordingly, all locations sampled have high densities of wild red deer, with the following mean density indices for the 5-yr period 1997–2001: Hareid 35.2 animals harvested annually per 10 km^2 qualifying area, Gaular 16.8, Nam-

sos 16.2, Eid 13.5, and Hitra 13.2 (Statistics Norway, 1998–2002).

Collection of samples

Liver samples were collected from 245 wild red deer during the hunting season between 10 September 2001 and 15 November 2001. The number of red deer examined from each municipality is shown in Table 1. A liver sample of approximately $5×5×5$ cm, was collected by the hunter, placed in a plastic box, and sent by mail to the laboratory as soon as possible after the animal was shot, together with information about location, date, sex, age group (calf, yearling [1.5 yr], or adult [≥2.5 yr]), body condition (good, moderate, or poor) on the basis of a general evaluation of the carcass, and any physical abnormalities. The hunters also gave information about the distribution of any mineral supplements ("salt licks") in the area in which the deer was shot. The liver samples were frozen at −20 C until analysis.

Chemical analyses

The trace element analyses were principally performed as described by Bernhoft et al. (2002). The Se analysis followed an accredited method (Norwegian Accreditation NS-EN ISO/IEC 17025), and the analysis of Cu and Co was carried out at an equivalent level of quality assurance. All elements were analyzed with the use of certified material SRM 1577 b (bovine liver from National Institute of Standards and Technology). The certified values (µg/g dry weight±SD) for Cu, Se, and Co were 160±8, 0.73±0.06, and 0.25 (indicative value), respectively. Our analyzed concentrations in this reference material for Cu, Se, and Co were 164±5 ($n=7$), 0.68±0.03 ($n=12$), and 0.25±0.02 ($n=8$) µg/g dry weight, respectively. The detection limit was 0.2 µg/g for Cu and 0.01 µg/g for Co and Se (wet weight).

For Cu and Co, the samples (1 g) were digested by a mixture of nitric acid and hydrogen peroxide in a closed system under pressure with microwaves. Samples were then diluted and analyzed by flame atomic spectroscopy (Cu), or flameless atomic spectroscopy with a mixed palladium-magnesium nitrate modifier (Co).

For Se, the samples (1 g) were digested by a mixture of nitric and perchloric acid on a programmed heating block. Selenium was then reduced to Se 4^+ and determined with a hydride atomic absorption spectroscopy system and sodium borohydride.

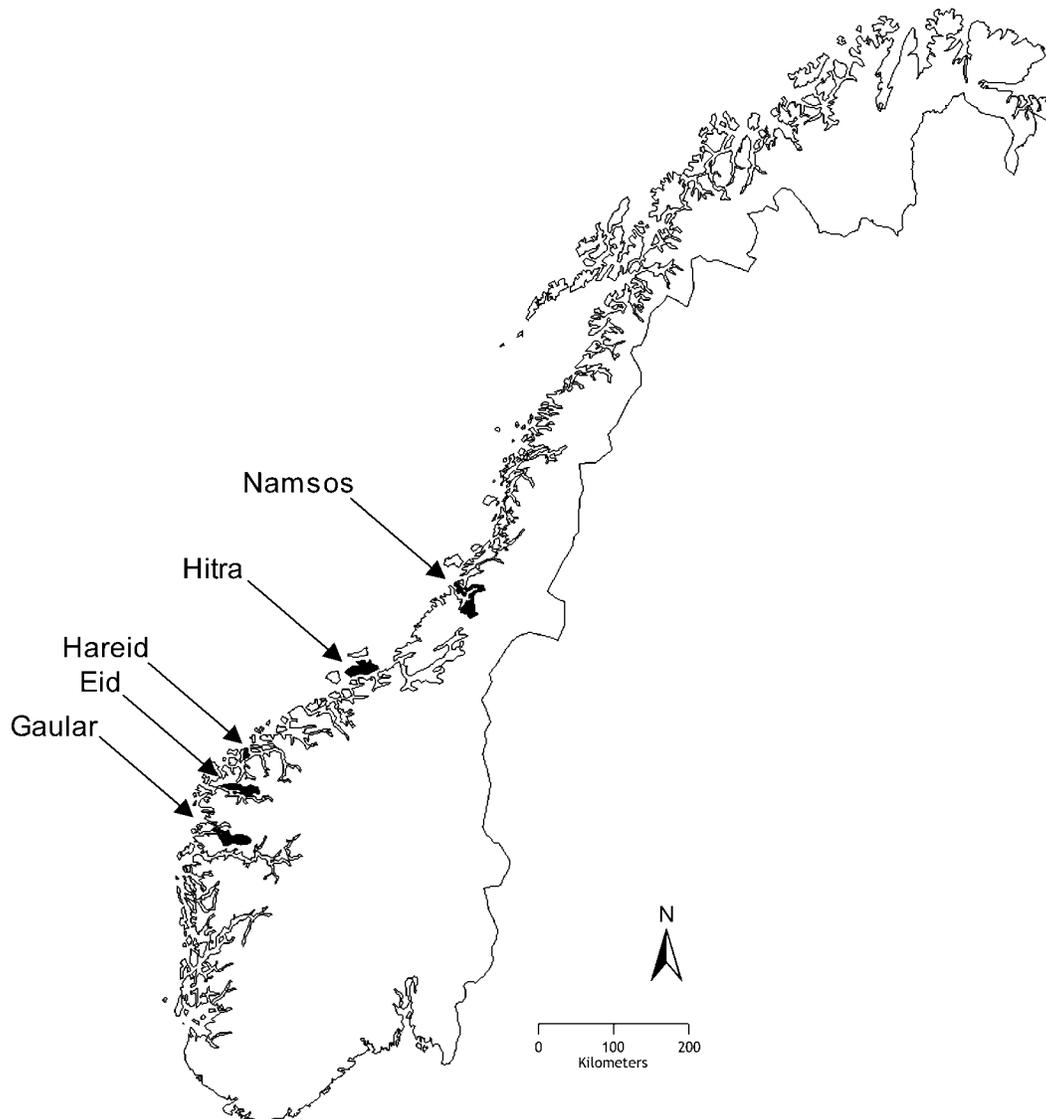All results are presented in wet weight (ww, µg/g).

FIGURE 1.   Map of Norway showing locations (municipalities) of the wild red deer (*Cervus elaphus*) sampled in 2001.

Statistical analyses

An analytical result below the detection limit (for Co only) was given a numeric value of half the detection limit for statistical calculations. Statistical calculations were performed with JMP 5.0.1 statistical software (SAS Institute Inc., Cary, North Carolina, USA). The level of significance was set at $P \leq 0.05$.

For testing of age group distribution between female and male red deer and the distributions of sex and age groups between geographic locations, contingency analysis with the Pearson form of the chi-square test was used.

Lack of normality in the distributions of element concentrations prompted the use of non-parametric methods in all statistical tests. The Kruskal-Wallis method was used for comparison of element concentrations in geographical locations and in age groups. The Wilcoxon rank sum method was employed for comparisons of element concentrations in groups with regard to sex.

RESULTS

The materials showed no significant difference in age distribution between female

TABLE 1. Liver concentrations of (µg/g wet weight) copper (Cu), cobalt (Co), and selenium (Se) in red deer (*Cervus elaphus*) shot during the 2001 hunting season in five different municipalities in Norway, according to locality and age group.[a]

Variable	n	Cu					Co					Se				
		Median	Mean	SD	Min	Max	Median	Mean	SD	Min	Max	Median	Mean	SD	Min	Max
Total	245	20	26	21	1.7	103	0.08	0.08	0.03	<DL	0.18	0.09	0.11	0.09	0.04	1.00
Locality[b]																
Hitra	45	6.4	12	12	2.7	69	0.08	0.07	0.02	0.04	0.16	0.11	0.16	0.12	0.04	0.50
Eid	49	17	22	17	1.7	84	0.07	0.07	0.02	<DL	0.12	0.07	0.07	0.02	0.04	0.14
Gaular	47	21	26	20	3.6	88	0.08	0.08	0.02	0.04	0.18	0.11	0.12	0.04	0.05	0.25
Hareid	52	23	27	20	3.4	91	0.08	0.07	0.02	0.02	0.12	0.10	0.12	0.13	0.05	1.00
Namsos	52	36	39	24	4.8	103	0.10	0.09	0.02	0.05	0.14	0.08	0.08	0.03	0.05	0.15
Age group[c]																
Calf	52	13	18	18	3.1	85	0.07	0.07	0.03	0.03	0.18	0.07	0.08	0.03	0.05	0.20
Yealing	80	15	21	20	2.7	103	0.08	0.08	0.02	0.04	0.14	0.08	0.10	0.05	0.04	0.37
Adult	112	29	33	21	1.7	102	0.08	0.08	0.02	<DL	0.16	0.10	0.13	0.11	0.05	1.00

[a] Min = minimum; Max = maximum; <DL = below the detection limit.
[b] Significantly different for all three elements: P<0.0001 for Cu, P<0.0001 for Co, and P<0.0001 for Se.
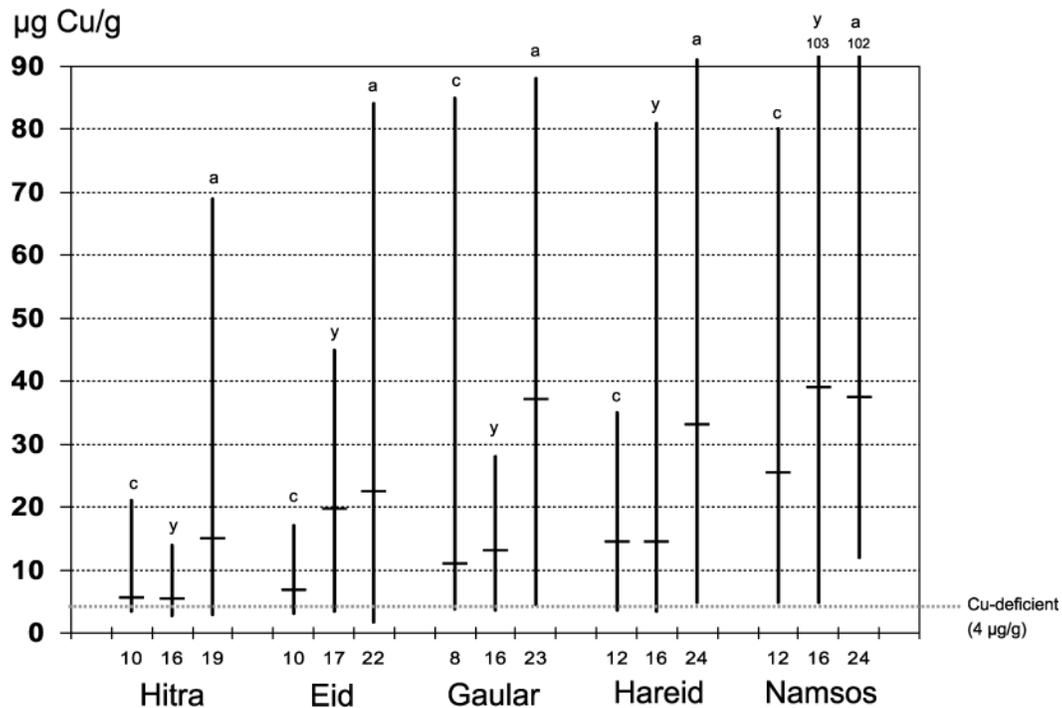[c] Significantly different for all three elements: P<0.0001 for Cu, P=0.04 for Co, and P<0.0001 for Se.

FIGURE 2.   Liver Cu concentrations (µg/g, wet weight) in various age groups of red deer (*Cervus elaphus*) shot during the hunting season in five Norwegian municipalities in 2001. Each vertical line represents one age group, showing the range (min–max) and the median (horizontal mark). The age category is marked at the top of each line: c = calves; y = yearlings; a = adults. The number below the bar shows the sample size in each age group.

and male red deer, and the distributions of sex and age were not significantly different between the geographic locations. The median liver concentrations of Cu, Co, and Se for all the examined deer were 20, 0.08, and 0.09 µg/g ww, respectively. The concentrations of Cu, Co, and Se at the various locations and for different age groups are presented in Table 1. For all three elements, liver concentrations differed significantly between the various locations (Table 1). The largest geographic variation was found for Cu, the median ranging from 6.4 (Hitra) to 36 µg/g (Namsos) (Table 1). We found significant differences in Cu, Co, and Se levels between age groups (Table 1). The median and range of Cu concentrations for calves, yearlings, and adults given for each location are shown in Figure 2. In the various age groups, no significant sex differences were found, with three exceptions: female

deer had significantly higher Co levels than males in the calf ($P=0.04$) and adult ($P=0.002$) age groups, and adult males had significantly higher Se levels than adult females ($P<0.0002$).

The individual liver Cu concentrations were compared with Cu reference values from farmed red deer given by Wilson and Grace (2001), which gave the following categories: deficient Cu (<4 µg/g ww), marginal Cu (4–6 µg/g ww), and adequate Cu (>6 µg/g ww). In total, 15 (6%) red deer—five calves, seven yearlings, and three adults—had Cu concentrations indicating deficiency, whereas 32 (13%)—10 calves, 17 yearlings, and five adults—had marginal Cu levels. Therefore, adequate Cu levels were found in 198 red deer (81%), which represented 93% of the adults, 70% of the yearlings, and 71% of the calves. The percentage of animals with deficient and marginal Cu concentration

was highest in Hitra, 13% and 36% respectively, followed by Eid, 8% and 10%, then Gaular, 4% and 11%, and finally Hareid, 6% and 6%. None of the red deer from Namsos were deficient, whereas 6% of them had marginal Cu levels.

The hunters reported 184 (75%) of the red deer to be in good condition and 60 (24%) to be in moderate condition. Only one was reported to be in poor condition, an adult male from Hareid with liver Cu levels of 69 μg/g, Co levels of 0.08 μg/g, and Se levels of 0.16 μg/g. According to the hunters, salt licks had been distributed in two hunting areas in Gaular, where three animals were sampled, and in one hunting area in Hitra, where a total of five animals were sampled.

DISCUSSION

Copper

Published data on liver Cu levels in wild red deer, as reported from different countries, are shown in Table 2. To compare our results given in wet weight (ww) with results in the literature given on a dry matter basis (dw), it has been assumed that liver has a mean dry matter content of 30% (Long, 1961). The lowest liver Cu values have been found in Germany; however, low Cu levels have also been reported from Poland, parts of Scotland, and Norway (Tables 1, 2). The individual Cu levels found in this study varied considerably; however, the levels were within the earlier reported range for Norwegian wild red deer (Table 2). We can assume, from the information given by the hunters, that the liver concentrations recorded are not influenced by the use of "salt licks" because only eight of the animals (3%) were shot in hunting areas where such licks were distributed. It can be concluded that the normal liver Cu concentration in wild Norwegian red deer seldom exceeds 100 μg/g ww during autumn. In comparison, approximately a twofold higher mean and maximum liver Cu level has been found in sheep from western Norway (Frøslie, 1977).

In this study, a significant geographic variation in Cu status of red deer along the west coast of Norway was documented. Similar geographic differences have been reported from other municipalities in this region (Kålås and Øyan, 1997). We found a 5.5-fold higher median Cu level in red deer from Namsos compared with Hitra. The mean red deer density index for the period 1997–2001 was higher in Namsos (16.2 animals harvested annually per 10 km^2 qualifying area) than in Hitra (13.2) (Statistics Norway, 1998–2002); thus, no indication of a direct correlation between high densities and low Cu concentration could be found (no statistics done). This was further supported by the fact that Hareid, showing the highest density index, had the second highest median Cu level in the study. The geographic variations in Cu status of red deer probably reflect variable dietary intake and absorption of Cu because of differences in available Cu from the soil and grazing plants in the local environment. Also, absorption and utilization of Cu in plants and animals is strongly influenced by the presence of Cu antagonists like molybdenum, sulphur, iron, zinc, and cadmium (Jones, 1994; Underwood and Suttle, 1999). Thus, the individual and geographic variation in Cu status of deer could also partly reflect variation in factors that interfere with the absorption and utilization of Cu by the animal.

The median Cu level found in Hitra is the lowest yet reported in wild red deer in Norway (Fig. 2; Tables 1, 2), and for calves and yearlings these values were <6 μg/g ww, the minimum concentration considered to be adequate in farmed red deer (Wilson and Grace, 2001). Low levels have also been found in red deer on Smøla, the neighboring island to Hitra, and in Vindafjord, located further south along the west coast (Table 2). The climate of the west coast of Norway is typically maritime, with favorable conditions for the formation of peat and soils rich in humus, which are known to be poor in several trace elements (Låg, 1975). The islands of Hitra and

TABLE 2.. Published data on liver copper (Cu) levels in free-ranging red deer (*Cervus elaphus*).[a]

Reference	Country[b]	*n*	Liver Cu (μg/g wet weight)			Comments
			Mean	Median	Range	
Frøslie et al., 1984	Norway (1)	5	15	NA	4–34	Smøla
Kålås and Myklebust, 1994[dw]	Norway (1)	58	16	11	1.2–73	Vindafjord
Calves		8	16	14	4.2–35	
Yearlings		12	8.2	5.2	1.2–28	
Adults		38	17	12	2.1–73	
Kålås and Øyan, 1997[dw]	Norway (5)					
Adult females (2.5–4.5 yr)		73	34	32	4.8–98	Including Vindafjord
Rosef et al., 2001	Norway (2)	43	44	39	0.8–112	Including Namsos
This work	Norway (5)	245	26	20	1.7–103	Table 1 for details
Cowie, 1976[dw]	Scotland (12)	37	11	7.6	0.9–63	
Adult females		31	7.4	3.6	0.9–33	
Adult males		6	29	28	11–63	
Barlow, 1980[dw, c]	Scotland (?)	289	NA	NA	0.4–79	
McTaggart et al., 1981[dw]	Scotland (6)	186	16	NA	NA	Rhum
Adult females		99	15	NA	NA	
Adult males		87	16	NA	NA	
Somlyay et al., 1983	Hungary (1)	NA	18	NA	NA	
Lusky et al., 1992	Germany (?)	26	NA	4.5	2.6–38	Winter-sampling
Hecht, 1996	Germany (?)					
<1 yr		94	NA	3.5	1.6–11[d]	
>1yr		381	NA	3.7	1.5–18[d]	
Falandysz, 1994	Poland (?)					
<2 yr[e]		55	7.8	NA	1.6–37	
Wolkers et al., 1994[dw]	Netherland (1)	50	22	NA	NA	
<0.6 yr		14	16	NA	NA	
0.6–1.5 yr		17	26	NA	NA	
1.5–5 yr		19	22	NA	NA	
Parker and Hamr, 2001[dw]	Canada (2)					
Fetus		4	135	NA	NA	
<1 yr		4	26	NA	NA	
≥2 yr		7	33	NA	NA	
Reid et al., 1980	New Zealand (>2)					
>6 mo		87	40	NA	NA	

[a] Entries marked dw have been calculated according to wet weight=dry weight/3.3 (Long, 1961).
[b] Number of locations/habitats examined in parentheses.
[c] The results were mainly based on the results of Cowie (1976) and McTaggart et al. (1981).
[d] The range is given as the 10%–90% percentile.
[e] The age was only suggested by the author.

Smøla are subject to an extreme maritime environment, and the makeup of the soil, for the most part, consists of peat.

A fifth of the examined wild red deer had inadequate Cu levels when compared with reference values for farmed deer (6 μg/g ww), which were surprisingly high, particularly considering that the sampling

was done in autumn when levels are anticipated to be at their highest (Wilson and Audigé, 1998). The hunters of the 15 red deer defined as Cu deficient (<4 µg/g ww) reported them to be either in good (13 deer) or moderate (2 deer) condition. An even lower Cu status was found in 37 well-fleshed feral red deer hinds from Scotland (Cowie, 1976), where 43% could be defined as Cu deficient (<4 µg/g). The low liver Cu status reported from Germany (Table 2; Lusky et al., 1992; Hecht, 1996) was not discussed by the authors from a deficiency point of view, and neither details about the time of sampling nor condition of the examined animals were given. However, the low levels found imply that red deer have the ability to adapt to low nutritional Cu levels. Available knowledge indicates that, despite having low Cu levels comparable to those found in farmed red deer suffering from emaciation, enzootic ataxia, osteochondrosis, or a combination of ills (<4 µg/g ww), wild red deer do not seem to develop these Cu-associated syndromes as far as can be discerned. Copper is essential for growth (Underwood and Suttle, 1999), and several studies show that growth might be restricted in young farmed deer with Cu deficiency (Wilson and Grace, 2001). Unsatisfactory growth rate was suggested, but not documented, in one of the sampled Scottish red deer populations low in Cu (Cowie, 1976). Thus, one must keep in mind that Cu deficiency leading to marginally reduced growth or vitality is easily overlooked in a wild population.

Studies of Cu status in farmed red deer in Norway have shown low levels. A median Cu liver concentration of 3.0 µg/g ww (n=52, range 1.0–21.8) was found in a survey including animals from 10 different red deer herds around the country (Rosef et al., 2001), which is considerably lower than the median Cu level (20 µg/g) found in wild red deer in this study. This implies that a decrease in Cu status might take place after capture and during farming, often reaching deficiency levels. In Norwe-

gian farms with deficiency problems like enzootic ataxia and osteochondrosis, affected red deer had liver Cu levels between 2.0 and 5.1 µg/g ww (Handeland and Flåøyen, 2000; Handeland and Bernhoft, 2004).

We found a significant age-related variation in Cu status among red deer. The adult age group had the highest median Cu level, both in the total material and at all locations, with the exception of Namsos (Table 1; Fig. 2). In Gaular, Hareid, and Hitra, the difference between calves/yearlings and adults was considerable. An age-related increase in liver Cu levels has also been documented for wild red deer in Canada (Parker and Hamr, 2001). However, the effect of age on normal Cu levels in wild red deer does not seem to be a straightforward "increase with age" scenario, because a general low Cu status within a red deer population seems to be reflected in the yearlings. In the low–Cu status locations of Hitra (Fig. 2) and Vindafjord (Table 2), the Cu levels in yearlings were as low as or even lower than those in calves and considerably lower than those in adults. It is to be expected that the metabolic requirements for Cu is high in calves and yearlings because of development and growth (Underwood and Suttle, 1999). Therefore, we suggest that in areas with low nutritional Cu level, Cu deficiency will occur primarily in the yearling and calf age classes.

Liver Cu concentrations were not significantly different by sex, which tallied with previous findings from Norway (Kålås and Myklebust, 1994), New Zealand (Reid et al., 1980), and Scotland (McTaggart et al., 1981). Cowie (1976) reported large differences in liver Cu status between hinds and stags in Scotland (Table 2), but the two groups were not from the same areas, nor were they sampled at the same time of the year.

Selenium

The median Se level found in this study was lower than previously reported in Nor-

wegian wild red deer (Frøslie et al., 1984; Kålås and Myklebust, 1994; Kålås and Øyan, 1997). However, Kålås and Øyan (1997) used a rather high detection level for Se of 0.5 μg/g dw (comparable to 0.15 μg/g ww), and the exact Se status could not be determined. To our knowledge, data on liver Se concentrations in wild red deer outside of Norway are extremely limited. Liver Se levels were measured in 139 white-tailed deer (*Odocoileus virginianus*) from eight different sites in Florida where pastures were known to be low in Se. Selenium levels ranged from 0.04 to 1.30 μg/g ww and had a mean of 0.20 μg/g (McDowell et al., 1995). In domestic ruminants, a hepatic Se concentration below 0.12 μg/g ww can be regarded as deficient (Van Metre and Callan, 2001); the median Se level found in red deer in this study was lower. Taking into consideration that the examined deer were sampled in an area of Norway in which the Se content of farmland soils has been found to be the highest (Wu and Låg, 1988), inland red deer could have an even lower Se status. Therefore, we conclude that the normal liver Se level in Norwegian red deer is low and could possibly represent a health problem. Young farmed red deer affected by white muscle disease had liver Se concentrations <0.035 μg/g ww (Wilson and Grace, 2001). This level is close to the minimum level of 0.04 μg/g found in two of the yearlings from this study (Table 1). At a Norwegian farm, three calves suffering from osteochondrosis and unthriftiness had low liver Se levels (0.04, 0.04, and 0.05 μg/g ww), in addition to low Cu levels, but no white muscle disease was diagnosed (Handeland and Bernhoft, 2004). In the majority of red deer farms submitting liver samples for the control of trace element status to the National Veterinary Institute, low concentrations of both Se an Cu have been found, indicating deficiencies and marginal status (unpubl. data).

Cobalt

Cobalt status is difficult to evaluate given the sparse information on normal liver levels in wild red deer. In red deer from Hungary, Somlyay et al. (1983) found a mean Co liver level of 0.92 μg/g ww, and Parker and Hamr (2001) reported a mean Co level of 0.57 μg/g ww in 11 red deer living in an area historically subjected to pollution from the copper-nickel-iron ore smelting industry in Canada. These levels are considerably higher than those found in our study. White liver disease is a well-known syndrome in lambs grazing areas with low Co along the west coast of Norway (Ulvund, 1990). Liver Co in healthy lambs is reported to be between 0.03 and 0.09 μg/g ww (Robertson, 1971), and in this study, individual red deer from both Eid and Hareid had Co levels below this. However, according to Jones (1994), evidence so far suggests that deer are not as susceptible to Co deficiency as other ruminants.

This study has shown that the Cu and Se status of wild Norwegian red deer is low in several areas in which the species has its major distribution. It is important to take this into consideration when capturing wild deer for farming purposes and to provide nutritional supplementation as needed. The significance of this finding, however, for the vitality and health of the wild red deer populations is unknown and needs to be explored further.

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