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Trace mineral status and liver and blood parameters in sheep without mineral supply compared to local roe deer (*Capreolus capreolus*) populations

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Abstract

General health, clinical-chemical blood analysis and copper (Cu), zinc (Zn), selenium (Se) and vitamin E concentrations in plasma and liver tissue (wet weight, ww) of two extensive grazing sheep flocks without mineral supply were compared to the status of local roe deer (*Capreolus capreolus*) populations (liver samples). Both sheep flocks were classified as healthy except for a remarkable variation in body weight and a slight foot rot infection in one flock. Hematology of sheep was normal, and total protein and creatinine as well as activities of creatin kinase, aspartat-amino-transferase, alkaline phosphatase and gamma-glutamyl-transferase in plasma were within reference levels. The mean of glutamate dehydrogenase (13.8 U/l) was slightly elevated in one flock. Mean liver concentrations of Zn (38.9 and 43.5 mg/kg ww) and Cu (111 and 87.5 mg/kg ww) in sheep flocks were higher compared to the respective roe deer populations (27.5 and 36.3 mg Zn/kg ww; 18.3 and 28.6 mg Cu/kg ww). This is supposed to be caused by differences in Cu and Zn metabolism in sheep and roe deer. Selenium deficiency was diagnosed in liver samples of both sheep flocks (0.21 and 0.23 mg/kg ww). There were neither significant differences compared to roe deer (0.21 and 0.27 mg Se/kg ww) nor differences depending on location. Correlations between plasma and liver concentrations of Cu, Zn and Se were not significant in sheep. Means of vitamin E in liver samples (30.6 and 41.8 mg/kg ww) were higher in roe deer populations. This may be caused by the opportunity of selective browsing for wild ruminants, which allows access to younger plants which are higher in vitamin E.

Keywords: Sheep; Roe deer; Copper; Zinc; Selenium; Vitamin E

1. Introduction

Imbalances in trace element metabolism are common in farm animals in north Europe (Sivertsen and Plassen, 2004; Govasmark et al., 2005; Humann-Ziehank and Ganter, 2006) and other countries like, e.g., Canada (Menzies et al., 2003). Deficiencies were frequently found in grazing sheep and goats, whereas overload appears once in a while resulting from disarrangement of food composition or, e.g., industrial pollution. In general, trace element deficiency remains without severe clinical signs for a long time. The main clinical signs may be bad body condition, reduced infection resistance and/or poor breeding results. The ability and effort of the owner

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to offer a commercial mineral supply is variable because of costs and presumed effort. Mineral supply prevails, but discussion regarding its necessity is common, particularly in some organic farming systems, due to the comparison to non-supplied and supposed healthy wild ruminants in the same region. The aim of this study is to evaluate the trace elements status of two extensive grazing sheep flocks without any mineral supply compared to the local roe deer population (*Capreolus capreolus*). The study was performed under field conditions, which did not allow to calculate the food composition and intake, especially in roe deer. Therefore, the results of the study should be utilized considering that differences due to dietary selection habits could not be quantified.

2. Material and methods

2.1. Farm animals

Two sheep flocks (Flock A, Blackhead sheep; Flock B, German Grey Heath Sheep), grazing in two different regions (A/B) in the north of Germany since spring 2005, were chosen for the study. The flocks were kept extensively and were fed with grass/herbage on pasture only during summer and autumn, without any additional mineral supply. The distance of the two regions was about 150 km. Sandy soil is predominant in both areas, the climate is mainly moderate. The plane landscape is a rural area with pastures, farmland and woodland in both regions, the difference in vegetation is low. Biomass at both pastures was suitable to provide sufficient food intake of the animals to keep physiological body weight conditions.

In November 2005, fifteen non-pregnant ewes from each flock were examined for general body condition and clinical health. The mean age of the animals was 3.0 years (S.D. 0.8) in Flock A and 2.7 years (S.D. 1.3) in flock B. Blood samples were taken from the jugular vein. As for plasma enzymes and Zinc (Zn), sampling was repeated once (enzymes) and twice (Zn), respectively, on the following 2 days. Enzyme activities and plasma Zn concentration in particular are known to stagger slightly from day to day. Therefore the average from the data was used for statistical analysis to minimize day-to-day variation. All additional parameters in blood were measured at day 1 only. Finally the ewes were sent to slaughter and liver samples were collected during slaughter processing.

2.2. Local roe deer population

During the same time period (autumn 2005) liver samples from local roe deer populations (*C. capreolus*) in regions A and B were collected following routine hunting. During the given time period four and eleven animals were shot in regions A and B, respectively. The samples were frozen at −20 °C until analysed. Selenium (Se), Zn, copper (Cu) and vitamin E were analysed in liver tissue.

2.3. Laboratory methods

Ethylene diamine tetraacetic acid (EDTA) was used as an anticoagulant for blood samples which were analysed immediately after sampling for packed cell volume, haemoglobin, erythrocytes and leucocytes by microcentrifugation, cyanohaemiglobin method and cell counter, respectively. Activities of creatine kinase, (CK), glutamate dehydrogenase (GLDH), gamma-glutamyl-transferase (GGT), alkaline phosphatase (AP) and aspartat amino transferase (ASAT) were measured in heparinized plasma by enzymatic UV-standard procedures using commercial test kits (Labor + Technik, Berlin, Germany). Total protein and creatinine were analysed by refractometry and enzymatic PAP test, respectively. Liver samples were prepared for analysis by wet digestion. All liver values are related to liver wet weight (ww) and were measured following wet digestion. Selenium was determined fluorometrically (Koh and Benson, 1983). Zink and Cu were analysed by atomic absorption spectroscopy. Vitamin E was analysed as α-tocopherol by HPLC (Rammel et al., 1983). Pooled samples of faeces from each flock were analysed for faecal egg count (FEC) by modified flotation, sedimentation procedures and the Bearmann method for the detection of lungworm infections.

2.4. Statistics

Data were analysed using SAS® statistical software (SAS Institute Inc., Carry, NC, USA). All parameters were checked for a Gaussian type of distribution (Shapiro–Wilks Test). In case of repetitive sampling (plasma enzymes/Zn, see above), the data were pooled for statistical analysis. Values of the different groups are usually presented as means ± standard deviations (S.D.). Comparisons of two independent animal groups were performed using the Student's *t*-test. Correlations between parameters were examined by calculating the Pearsson's Correlation Coefficient. The influence of the location factor was tested by an analysis of variance.

Median values and interquartile ranges are presented in parameters with a non-Gaussian type of distribution. For these parameters differences between groups were evaluated using the Wilcoxon's Two Sample Test and correlations between parameters were examined by calculating the Spearman's Correlation Coefficients.

P-values <0.05 were considered to be statistically significant.

3. Results

3.1. Clinical examination of sheep

Clinical examination of the sheep did not show distinct health problems except lameness in one ewe caused by a food root infection of flock A. There was a high variation in body weight in Flock A (mean: 53.2 kg, span 26 kg).

Table 1
Results of haematological and clinical chemical parameters of blood in the two sheep flocks

Flock	Haemoglobin (mg/l)	Packed cell volume (l/l)	Erythrocyts (T/l)	Leucocytes (G/l)	Total protein (g/l)	Creatinine (μmol/l)
A	118.6 ± 11.3	0.33 ± 0.03	11.2 ± 1.0	6.6 ± 2.0	71.1 ± 5.7	84.9 ± 17.8
B	115.8 ± 8.7	0.33 ± 0.02	11.1 ± 0.8	6.8 ± 1.8	67.6 ± 4.1	89.0 ± 16.7

Values represent the mean ± S.D. for $n = 15$. Differences between flocks are not significant.

Table 2
Results of analysis of plasma enzyme activities in the two sheep flocks

Flock	Creatine kinase (U/l)[a]	Aspartat aminotransferase (U/l)	Glutamate dehydrogenase (U/l)	Alkaline phosphatase (U/l)	Gamma-glutamyl transferase (U/l)
A	73.5 (36–97.5)	47.1 ± 6.8[b]	13.8 ± 4.2[c]	–	27.1 ± 10
B	37 (17.5–148)	60.0 ± 14.1	6.6 ± 2.1	6.84 ± 1.8	24.6 ± 5.3

Values represent the mean ± S.D. for $n = 15$; Alkaline phosphatase was not measured in flock A.
[a] Values represent median and interquartile range because of a non-Gaussian type of distribution.
[b] $P < 0.05$ vs. sheep flock B.
[c] $P < 0.01$ vs. sheep flock B.

Table 3
Trace elements in plasma of two sheep flocks (A/B)

Flock	Copper (mg/l)	Selenium (mg/l)	Zinc (mg/l)	Vitamin E (mg/l)
Flock A	17.6 ± 3.2[a]	0.051 ± 0.012	0.53 ± 0.06[a]	2.0 ± 0.5[a]
Flock B	20.2 ± 2.6	0.055 ± 0.015	0.59 ± 0.07	3.3 ± 0.7

Values represent the mean ± S.D. for $n = 15$.
[a] $P < 0.05$ vs. sheep flock B.

3.2. Results of laboratory examinations

Haemoglobin, packed cell volume (PCV), leucocytes, erythrocytes, total plasma protein and creatinine, as well as activities of CK, ASAT, AP and GGT were within reference values for sheep/within the norm (Bickhardt and Konig, 1985) in both sheep flocks. The mean of GLDH was slightly above the upper reference level of 12 U/l. Means and standard deviations are given in Tables 1 and 2.

Control of endoparasites in pooled samples of faeces showed a mild worm burden: in herd A 7 strongyle eggs/g were found. Moreover, control of small lung worm was positive. In herd B there were 38 strongyle eggs/g; 7 eggs of *Strongyloides papillosus* were detected. Furthermore, *Trichuris spp* was positive.

The results of analysis of trace elements in plasma of sheep are shown in Table 3. Trace element concentration of liver samples from sheep and roe deer are given in Table 4. The comparison of mean liver concentration of Cu, Zn, Se and vitamin E in the sheep flocks regarding the influence of location showed a higher level of vitamin E ($p < 0.05$) in sheep in flock B. There were no significant correlations between plasma and liver concentration of Cu, Zn and Se in sheep, whereas vitamin E concentrations in liver and plasma were well correlated

Table 4
Trace elements in liver tissue (wet weight) of two sheep flocks (A/B) and two roe deer groups

Group	Copper (mg/kg)	Selenium (mg/kg)	Zinc[a] (mg/kg)	Vitamin E (mg/kg)
Flock A ($n = 15$)	111 ± 36.8[b]	0.21 ± 0.04	38.9 (36.3–48.4)[b]	12.1 ± 2.7[b,c]
Flock B ($n = 15$)	87.5 ± 51.2[b]	0.23 ± 0.04	43.5 (39.5–44.8)[b]	17.6 ± 5.9[d]
Roe deer A ($n = 4$)	18.3 ± 12.0	0.21 ± 0.04	27.5 (26.3–30.0)[c]	30.6 ± 14.3
Roe deer B ($n = 11$)	28.6 ± 21.2	0.27 ± 0.07	36.3 (33.8–38.3)	41.8 ± 14.1

Values represent the mean ± S.D.
[a] Values represent median and interquartile range because of a non-Gaussian type of distribution of this parameter in flock A.
[b] $P < 0.05$ vs. roe deer data in the same column and identical area.
[c] $P < 0.05$ vs. the same species from the other area.
[d] $P < 0.01$ vs. roe deer data in the same column and identical area.

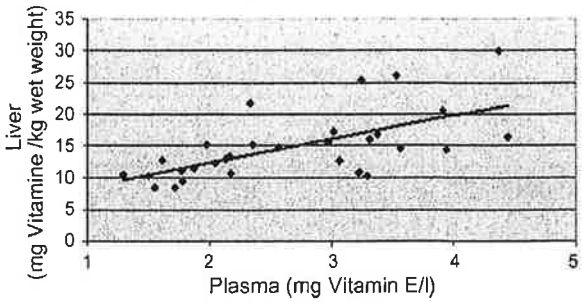

Fig. 1. Correlation between vitamin E concentration in plasma and liver in sheep; $n = 30$; $y = 3.7356x + 4.9065$; $r = 0.63467$.

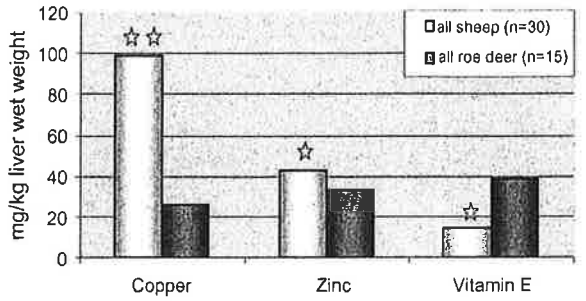

Fig. 2. Comparison of means of copper, zinc and vitamin E concentration in liver of all sheep (flock A + B) vs. all roe deer, disregarding location (*/** $p < 0.05/0.01$).

(Fig. 1). The comparison of mean concentrations of Cu, Zn and vitamin E in liver tissue of all sheep ($n = 30$) vs. data of all roe deer ($n = 15$), disregarding location, turned out significant differences (Fig. 2); so, there were higher concentrations of Zn and Cu in sheep, whereas vitamin E was higher in roe deer. The combined illustration in Fig. 2 is considered to be suitable in this case because of mostly congruent reference levels of both species. Therefore, the figure may offer a better understanding of differences between species.

Mean and S.D. of Se concentration in liver tissue of all sheep and all roe deer was 0.217 ± 0.039 mg/kg FS and 0.252 ± 0.072, respectively. The difference was not significant.

4. Discussion

Generally, the results of this study demand careful evaluation considering the experimental conditions as mentioned above.

Examination and haematological/biochemical analysis of blood samples result in the assertion that both sheep flocks can be regarded as clinically healthy except for a remarkable variation of body weight and a scattered foot rot infection in flock A. Faecal egg counts were within tolerant ranges for sheep at pasture. There was no distinct elevation of the enzyme activities of GLDH, CK and ASAT, which is common by cases of clinical manifestation of selenium and/or vitamin E deficiency in sheep (Bickhardt et al., 1999). Determination of Glutathion peroxidase was not considered, because this enzyme seemed to be unsuitable for the Se status in sheep (Bickhardt et al., 1999).

There were no typical clinical signs of Zn deficiency like parakeratosis, dermatitis and wool loss (Hennig-Pauka et al., 2001). Alkaline phosphatase, which may be low in Zn-deprived animals (Underwood and Suttle, 1999) was within reference levels. Signs of Cu deficiency which are predominant in lambs showing signs of defective enervation of the muscular system (sway back) had not been reported previously in both flocks.

In general, it is not easy to assess trace element status in livestock. As in our case, it is often impractical to determine dietary composition or amounts consumed such as in the pasture feeding situation. Frequently, trace element status in mammalians is diagnosed using blood analysis. This is assumed to be adequate in case of Se, but blood analysis of Zn is an insensitive measure of Zn status due to several non-nutritional sources of variation of serum Zn like stress and inflammation (Herdt et al., 2000). Plasma Cu concentration within the range of 3–9 μmol/l may generally indicate a stage of marginal deficiency. However, plasma Cu may not accurately reflect Cu status in livestock in general as normal or high plasma Cu concentrations do not necessarily indicate that the animal has a sufficient amount of Cu. Investigations into chronic Cu poisoning in sheep showed normal or even low plasma Cu concentrations in poisoned animals (Humann-Ziehank et al., 2001). At present the best method to monitor the Cu status in livestock requires liver tissue (Herdt et al., 2000). In sheep livers concentrations of 1–7 mg Cu/kg fresh weight can probably coincide the marginally deficient state (Underwood and Suttle, 1999).

There have been very few studies undertaken on reference values in order to assess the trace element status of wild ruminants under natural conditions. Most published papers are related to questions of industrial pollution. There are data for farmed red deer and fallow deer (Puls, 1994; Wilson and Grace, 2001). In Germany some collected data from long-term studies of heavy metals in soil–plant–wild animals (Hecht, 1994) do exist, but no reliable reference values.

4.1. Copper

Mean liver Cu values of both sheep flocks remain within physiological values (Puls, 1994; Humann-

Ziehank et al., 2001) of 20–120 mg Cu/mg ww. Noteworthy was the enormous span of values with standard deviations of 36.8 and 51.2 mg Cu/kg ww in flock A and B, respectively. This seems to underline high inter-individual differences in Cu storage, which was found earlier in studies on Cu metabolism in sheep (Humann-Ziehank et al., 2001). In conclusion, for assessing Cu status of a flock a sufficient number of sampled animals has to be considered. Plasma Cu concentration was within reference values for sheep of 7–24 μmol/l (Bickhardt and Konig, 1985), but no correlation with liver Cu concentration was found as described earlier (Herdt et al., 2000; Humann-Ziehank et al., 2001).

The lack of reference values for roe deer under local conditions demands careful validation of the results. The mean liver Cu concentration of roe deer look similar to results of previous examined roe deer populations in Germany (Hecht, 1994) with mean values of 10–20 mg Cu/kg ww. Studies of other wild ruminants suggest values above 6.4 mg Cu/kg liver ww to be adequate for red deer (Grace and Wilson, 2002). Reference values published for fallow deer and red deer are 25–80 and 20–120 Cu/kg liver ww, respectively (Puls, 1994). Therefore, Cu status of both roe deer populations may be settled at the lower limit of normal range. However, a wide difference was found between the Cu status of sheep and roe deer at the same location with much higher liver Cu concentrations in sheep (Table 4). Moreover, comparison of mean Cu content in liver of all sheep vs. all roe deer, disregarding location, leads to the assumption that there may be differences in Cu storage of the two species.

4.2. Zinc

The mean plasma Zn concentrations of both sheep flocks were below the reference values of 0.80–1.20 mg/l, whereas Zn concentration in liver tissue was within reference ranges (Puls, 1994) of 30–75 mg/kg ww. Moreover, no correlation between liver and plasma concentrations could be demonstrated. Inflammatory mediators are known to stimulate the transfer of Zn out of the plasma into tissue pools (Bremner and Beattie, 1990), but clinical and biochemical results did not indicate inflammation in our case. Liver and plasma Zn concentrations require a careful interpretation and may point out a marginal Zn status for sheep in both flocks.

Zinc status of the respective group of roe deer at the same location was significantly lower compared to the sheep flocks. Moreover, there were significant differences between the roe deer groups at the two different locations (Table 4). Disregarding location the mean of all sheep vs. the mean of all roe deer showed significantly higher Zn status in sheep. Mean liver Zn concentrations of 26.1 and 31.5 mg/kg ww in German roe deer populations were described earlier (Hecht, 1994). In comparison to reference values (Puls, 1994) of fallow deer (30–60 mg/kg ww) and red deer (23–80 mg/kg ww) the Zn status seems to be at a low but sufficient level.

An investigation into mineral content of leaves (Rahmann, 2004) from 16 different bushes and trees in pastures in north Germany showed that there were only four shrubs species with higher Zn concentrations in leaves than mean Zn content of grass at pasture. Therefore, the roe deer population may not have a noticeable advantage from selective ingestion of leaves for example.

4.3. Selenium

Mean Se concentration in plasma and liver was below reference values (Puls, 1994) of 0.08–0.5 mg/l and 0.25–1.5 mg Se/kg liver ww in both sheep flocks, respectively. There were neither significant differences compared to roe deer nor differences depending on location. This confirms the assumption of general low Se content at pasture in the north of Germany (Boehnke et al., 1997; Bickhardt et al., 1999) and other north European countries (Govasmark et al., 2005). Although plasma Se levels are indicated to reflect the Se status of livestock in literature (Herdt et al., 2000), no correlation to liver Se levels could be found in our data. Commonly, a linear relationship between plasma and liver Se levels can be demonstrated (Valimaki et al., 1987), but that may fail in cases of very low Se concentrations analysed in our animals.

Normal Se levels in red and fallow deer are indicated at the same levels as for sheep (Puls, 1994). Therefore, Se status in both roe deer groups may be characterised as low as well. The inclusion of roe deer in marginal Se provision may indicate in general low Se levels in plants consumed by roe deer in this area. Selenium is a non-essential trace element for plants. The random investigation of Rahmann (2004) into mineral content of leaves from bushes and trees at pasture in north Germany resulted in only one plant showing higher Se content of leaves compared to grass on pasture.

4.4. Vitamin E

Vitamin E status of both sheep flocks was within reference levels (Puls, 1994) of >10 mg/kg liver ww and 1–5 mg/l plasma. The significant correlation between plasma and liver concentration (Fig. 1) proves reliability of both materials of diagnostics. The significant differ-

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ences between the two sheep flocks (Table 3) may be explained by keeping conditions. Flock A was kept at one fenced pasture for months, whereas flock B were able to move more often in the same area. Therefore, flock B had much more access to fresh grass. Vitamin E is instable in forages and content depends on season and duration of storage. Grass on pasture in autumn is known to decline in vitamin E concentration. The typical time for clinical vitamin E deficiency is late winter when feeding consists of residuary hay.

The much better vitamin E status of the roe deer at the same location (Table 3) as well as when disregarding location (Fig. 2) may be explained by the much more selective browsing behaviour of wild ruminants. This enables taking of buds, fresh grass or leaves with higher vitamin E content. The status of two roe deer groups was close to data of an earlier study (Haacker, 1975) on female roe deer showing 40.3 ± 18 mg vitamin E/kg liver ww. In addition, those animals showed distinct individual differences in vitamin E status as well as season-dependent variation with highest concentrations in summer.

5. Conclusions

In conclusion, we demonstrated that general low Se content in plants can result in Se deficiency in sheep flocks and roe deer populations just as well. Clinical effects may arise in stress situation like infection, birth or physical exercise. Therefore, preventive Se supply in sheep flocks is recommendable and it may be advantageous for roe deer as well. Storage of Cu and Zn in the liver seems to be more effective in sheep compared to roe deer. Due to the opportunity of selective browsing, only vitamin E supply is advantageous in roe deer compared to sheep, whereas Se, Cu and Zn are unaffected or even lower. But additional studies on trace element metabolism in roe deer are necessary to verify, if this is a species specific effect. The fact that clinical signs of Se deficiency have not yet been described in roe deer may be explained by the good vitamin E supply. Vitamin E and Se serve as antioxidant and severe clinical signs are described particularly in the combined Se and vitamin E deficiency in small ruminants (Bickhardt et al., 1999).

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