Copper deficiency in yak (bos grunniens) at whipsnade wild animal park

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Abstract
Copper deficiency was suspected in the herd of yak (Bos grunniens) at Whipsnade Wild Animal Park. Animals were suffering from a range of conditions, including chronic diarrhoea, poor body condition and dull coats, despite daily supplementation with 36 mg of copper per animal. The copper concentration in yak blood samples collected in 1994 ranged from 0.8 to 3.3 μmol/L (mean 1.8, n = 10), well below the normal cattle range of 12 to 19 μmol/L. The copper supplement was therefore increased to 720 mg copper per adult per day (1.8 g copper sulphate) and blood copper concentrations rose each year, apart from low concentrations in juveniles in 1998, to plateau between 8.1 and 20.1 μmol/L (mean 13.3, n = 21). Over the same period general body and coat condition improved, again with the exception of 1998, and herd size grew. Other factors which may have been involved in the improvement in the health of the herd were the importation of a new herd male in 1992, and an intensification of anthelminthic treatment over the same period.

Keywords
yak, Bos grunniens, copper, molybdenum, parasitism, fertility, depigmentation

1 Introduction

Copper is an essential mineral for tissue oxidation, and a deficiency can cause disorders of keratinization, formation of melanin, elastin and haemoglobin, and reduced osteoblastic activity (Radostits et al. 1994). Typical clinical signs include retarded growth, poor quality and/or depigmented fur or wool, diarrhoea, anaemia, and ataxia. Reduced fertility and immunity have also been ascribed to copper deficiency in some species.

The supplementation of zoo diets with copper has been found to improve fertility, reduce the number of stillbirths and immature offspring, and inten-
ify rutting behaviour in several ungulate groups (Senf & Zscheile 1978). For one species, the blesbok (Damaliscus dorcas phillipsi) a deficiency state has been demonstrated both in captivity (Jones 1980) and in the wild (Turkstra et al. 1978), and this may reflect an unusually high requirement for the element (Dierenfeld et al. 1988). A condition resembling enzootic ataxia has been described in yak (Bos grunniens) (Whitehead 1950), but no diagnostic investigations were reported. However, Liu, Zhang and Huang (1995) found very low liver copper concentrations in apparently healthy yak, and Claus and Dierenfeld (1999) reported on clinical deficiency in yak, and presented evidence for a particular susceptibility to deficiency of the element.

Copper deficiency has been diagnosed, or suspected, in a number of ruminants at Whipsnade Wild Animal Park including bontebok (Damaliscus dorcas) (Jones 1980), llama (Llama glama) (Palmer et al, 1980), European bison (Bison bonasus), wapiti (Cervus elaphus) and yak (Ashton et al. 1979). Clinical signs recorded in yak included debility, weight loss, anaemia, diarrhoea, hindleg ataxia, exercise intolerance, alopecia, depigmentation around the eyes, stillbirths and poor neonatal survival. Between 1972 and 1979, 43 yak serum samples were analysed and found to contain between 10 and 100 μg/dl copper, with a mean of 40 (1.6 to 15.7 μmol/l; mean 6.2, Ashton et al. 1979). During the same period the liver copper concentration of four yak ranged from 8.5 to 26 μg/g dry matter (DM), with a mean of 13.4 (133 to 408 μmol/kg DM; mean of 210). Both means were below the normal copper ranges for cattle established by the Veterinary Laboratories Agency, UK: 9–19 μmol/l serum and 300–8000 μmol/kg DM liver. A grass sample from the yaks’ paddock contained 10.6 mg copper/kg DM (166.4 μmol/kg DM), thought to be a mildly inadequate concentration, while repeated, seasonal testing of six paddocks between 1983 and 1984 for molybdenum, known to interfere with copper absorption, only revealed one sample out of 48 with a concentration of molybdenum above 2.0 mg/kg DM (2.3 mg/kg DM, all others were below 1.5 mg/kg DM). Between 1979 and 1992 the yak diet was supplemented with copper sulphate, but the inclusion rate was variable and never greater than 180 mg copper per adult per day.

Between 1992 and 1994 it was evident that yak were still suffering from a range of conditions which could be due to copper deficiency. We present here the results of opportunistic investigations of the copper status of the herd before and after increases in dietary supplementation.

2 Materials and Methods

Yak were introduced to Whipsnade in 1944 and have been kept as a closed herd with occasional new animals. The only arrival in the last 20 years was a new breeding male imported from Sweden in 1992. Details of individuals and the herd population were taken from animal management records which since 1989 have been stored on the ARKS database (International Species Information System, Apple Valley, MN, USA).
From 1990 to the present day the yak were kept in a large grassed drive-through exhibit on a dense loam (limestone and clay) soil, which they shared with Bactrian camels (*Camelus bactrianus*) and Père David's deer (*Elaphurus davidianus*). They had permanent access to shelters and, during the winter, were housed at night. Supplementary hay was offered during the winter, but in the winter of 1998/1999 silage was fed, and in 1999–2000 haylage. Approximately 1.25 kg of grazer concentrate pellets (14% protein) were offered per adult per day. Up to 1994 these contained 21 mg/kg (Zoo Grazer G. P. Diet) and since 1994 25 mg/kg (Whipsnade Grazer). Copper sulphate was supplemented daily, applied to the concentrate pellets as an aqueous solution, and allowed to soak in before feeding exclusively to the yak. Between 1991 and 1994 40 ml of a 2.5% solution was given to 11 animals, or approximately 36 mg of copper per animal. In June 1994 the supplement was increased to 144 mg per animal per day, the same rate as used in 1979, and then after analysis of blood results (see below) increased further to 720 mg per animal per day from September 1994. The allowance for calves was half of the adult dose until they were over one year of age. Anthelmintic drugs were administered in the feed three times yearly (April, July and September). Prior to 1995 fenbendazole (Panacur 1.5% pellets) was offered as an addition to normal food on one day, but from 1995 onwards fenbendazole (Panacur 4% powder) and ivermectin (Ivomec Premix for Pigs) were used on alternate years, incorporated into the concentrate feed and fed for three consecutive days.

Jugular venous blood samples were collected from yak immobilized for any management or veterinary procedure and stored with lithium heparin. This routine testing started in 1994, therefore the small numbers of samples tested between 1989 and 1993 were not analysed. Liver samples were collected during routine *post mortem* examinations of animals which died or were euthanased. Both were submitted to laboratories of the Veterinary Investigation Service, formerly of the Ministry of Agriculture, Fisheries and Food, and latterly the Veterinary Laboratories Agency. The copper content of the whole, heparinised, blood was determined by graphite furnace atomic absorption spectrophotometry using a GBC 906 AAS at 324.7 nm following a sample dilution of 50 μl with 1.00 ml of 0.05% Triton-X-100 diluent. The copper content of liver samples was measured by flame atomic absorption spectrophotometry using a GBC 908 AAS, after sample dissolution in nitric acid. Blood values were compared with the normal range for cattle (whole, heparinised blood) established by the VLA: 12–19 μmol/l. Grass was collected from the enclosure in 1994, and submitted for analysis at the Royal Veterinary College, North Mymms, and again in 1998 when it was analysed at the VLA, Sutton Bonington by flame atomic absorption spectrophotometry using a GBC 932 AAS after digestion with nitric, perchloric and sulphuric acids. Unfortunately, conserved forages were not tested.
3 Results

3.1 Clinical cases

In 1992 several cases of diarrhoea were recorded in the yak herd, and two individuals, a 17-year-old female and a sub-adult male, were euthanased because of the chronic nature of the diarrhoea and loss of body condition. The copper concentration of the female's liver was 132 µmol/kg DM, less than half of the low end of the normal cattle range. In 1994 a sub-adult female, approximately 18 months old, was euthanased due to chronic hindlimb ataxia and poor body condition. The copper concentration of her liver was similarly low; 162 µmol/kg DM.

Since 1994 the general body condition of the herd has improved, and it has been noticeable that coat colour is now black, with some grey in older animals, whereas there used to be a brownish tinge. Recurrent diarrhoea, as noted in 1992 and previous years, has not been seen since. There are still cases of diarrhoea, but they are isolated cases for which a cause can normally be established, for example gastro-intestinal parasitism.

In the autumn of 1998 the calves born during the summer had depigmentation of the hair around the eyes, but were otherwise in good health. Their blood copper concentrations ranged from 0.8 to 5.0 µmol/L (mean 2.8), and the four male calves, which were culled, also had extremely low liver copper concentrations (17 to 135 µmol/kg DM, mean 73), plus moderate gastrointestinal nematode burdens (50-3550 abomasal worms, mean 2350, and 700 to 4350 small intestinal worms, mean 2950). The female calves were injected intra-muscularly with 62.5 mg copper heptonate (Cuvine) and sub-cutaneously with 0.2 mg/kg body-weight ivermectin (Ivomec), and no further clinical signs were seen. A calf born in 2000 was euthanased at three months of age because of poor growth. Its liver copper concentration (366 µmol/kg DM) was within the normal cattle range, but at the low end of the range.

3.2 Blood copper results

The copper concentration in blood samples taken from animals immobilised for clinical or management purposes in 1994 ranged from 0.8 to 3.3 µmol/L (mean 1.8, n = 10), well below the normal cattle range of 12 to 19 µmol/L (figure 1). The copper supplement was increased in September 1994, and over the period 1995-2000 blood copper concentrations rose steadily, apart from 1998, to plateau in 1999-2000 between 8.1 and 20.1 µmol/L (mean 13.3, n = 21).

The low concentrations in 1998 were primarily those of the ten calves born during the summer and tested in the autumn. Two yearlings which were examined at the same time also had low concentrations (0.5 and 1.3 µmol/L), but three adult females sampled during the year had much higher concentrations (8.4, 17.0 and 18.1 µmol/L).
Fig. 1. Yak blood copper concentrations (1994–2000).

3.3 Liver copper concentrations

The liver concentrations of adult yak which died between 1995 and 2000 ranged from 557 to 4808 μmol/kg DM (mean 3125, n = 5), all within the normal cattle range. Ten neonates which were culled in 1999 had liver copper concentrations between 1699 and 6343 μmol/kg DM (mean 3948), also well within this range. However, the four male calves culled in 1998 had low values (see above) and a yearling female culled earlier in 1998 was also deficient (168 μmol/kg DM).

3.4 Pasture analysis

In 1994 the grass in the yak paddock was found to contain 8.3 mg copper/kg DM (130 μmol/kg DM). A further check in 1999 revealed a concentration of 19.7 mg/kg DM (309 μmol/kg DM).

3.5 Breeding

Breeding in the yak herd from 1988 remained fairly static at between two and four calves per year until after the introduction of the new male in 1992. The following year there were five calves born (figure 2), but in 1994 only two. However, from 1995 onwards the number of births increased each year until population control measures had to be taken in 1999 and 2000. The number of deaths peaked in 1988 and 1992, but in all other years were less than three, consequently the population grew from 1995 and animals were exported or culled each year except 1996.

Fertility was assessed as the number of live calves produced by female yak older than 1.5 years-old on the 1st January of that year, and still present during the calving period (March to August). This percentage was 60 % or greater in every year from 1990 to 2000 except one, 1994 (figure 3).
4 Discussion

Increased supplementation with copper sulphate, either alone or in combination with other changes in husbandry, such as the importation of the new male and intensification of anthelmintic treatment, led to a marked improvement in the health and reproduction of the yak herd at Whipsnade. Opportunistic blood sampling provided the evidence that blood copper values in the yak rose simultaneously with these improvements, but because of the limitations of a clinical study it was not possible to obtain enough samples to prove cause and effect. Also, it was not possible to perform enough haematological examinations to study the association between red blood cell parameters and copper concentration, and to compare with yak results from the 1970s and early 1980s (Hawkey et al. 1983).
The diet offered to the yak between 1992 and 1994 was thought to contain sufficient copper. The minimum requirements for cattle are 10 mg copper per kg dry matter (Radostits et al. 1994) and, although the grass concentration in 1994 was slightly below this figure, the concentrate pellets (21–25 mg/kg) and existing supplement should have compensated. Moreover, at this stage all ages of animals, and dominant as well as lower-ranking individuals, had low blood copper concentrations, suggesting that lack of access to concentrates was not an important factor. Instead, there was probably a combination of secondary deficiency, due to one or more factors reducing the absorption of copper, and a uniquely high requirement by yak for the element (Clauss & Dierenfeld 1999). Neither the bactrian camels nor the Père David’s deer sharing the pasture, but not receiving any supplement, were affected in the same way, underlining the particular sensitivity of yak. The final dose of copper supplement which was necessary for effective prophylaxis, 720 mg per animal per day, was consistent, on a comparative weight basis, with the daily figure of 1200 mg required for cows suffering from molybdenum-induced secondary deficiency (Radostits et al. 1994).

The conditioning factor for secondary copper deficiency is often not known, but the most commonly identified factor is an excessive dietary intake of molybdenum. This appears unlikely in this situation because extensive testing in the 1980s failed to show any elevation of the element. Other elements, such as sulphur and iron, have not been tested, but the paddock used by the yak is grazed very heavily, and so it is likely that animals do ingest soil as they graze.

Two other factors which could potentially have acted over the same time period were the importation of a new herd male in 1992, and an intensification of anthelminthic treatment over the same period. The new male was responsible for introducing new genes into the in-bred herd and undoubtedly this helped improve reproductive success, but this improvement was mainly apparent from 1995 after the copper supplement was increased, and not from 1993 when the new male’s first offspring were born. The parasite control protocol for all ungulates was reviewed in 1994, at the same time as the review of copper supplementation, and the treatment of the yak was incorporated into the concentrates, and given over three days to ensure that all individuals received a therapeutic dose. However, the impact of gastro-intestinal parasitism is difficult to assess. Both parasitism and copper deficiency affect the same individuals, primarily the young, and both were treated in the food, and so young and sub-dominant animals would have had reduced access to anthelminthic drug and copper sulphate. There is evidence that the two interact: intestinal nematodes may reduce the absorption of copper, but copper supplementation has an anthelminthic effect (Sykes 1987, Poppi et al. 1990). Copper deficiency is also thought to reduce an individual’s immune response. It was notable, therefore, that the calves which showed signs of copper deficiency in 1998 also had moderate nematode infections.

There was not a clear-cut association between low blood copper concentrations and fertility, although the very low figure in 1994 reflected the poor
health and low copper status at the time, and there was an increase in parallel with copper supplementation between 1995 and 1998, with a dip in 1999 possibly caused by the copper deficiency seen in juveniles the previous year. Fertility was 60% or greater during the period 1990–1993, but unfortunately comparable herd blood copper concentrations were unavailable. The herd did increase slightly in size over this period, but the main increase occurred from 1995 onwards when there were more births each year, but no comparable increase in mortality.

5 Conclusions

1. Successful treatment of copper deficiency in yak required large doses of copper sulphate. Supplementation has been continued for several years.

2. The copper concentration of whole blood samples, collected whenever animals were immobilised, reflected the copper status of the herd, and indicated a deficiency in juveniles despite supplementation.

3. Improvements in herd reproductive success may require correction of several factors, including genetic inbreeding and diseases such as copper deficiency and gastrointestinal parasitism.

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Products mentioned in the text

Cuvine: copper heptonate, 12.5 mg/ml, injectable copper supplement, manufactured by Vericore, Littlington, Herts., UK.

Ivomec Injection for Cattle: ivermectin 10 mg/ml, injectable anthelmintic, manufactured by Merial Animal Health Ltd., Harlow, Essex, UK.

Ivomec Premix for Pigs: ivermectin 0.6 %, manufactured by Merial Animal Health Ltd.

Panacur 1.5 % pellets: fenbendazole 1.5 % oral pellets, manufactured by Intervet UK Ltd., Milton Keynes, Bucks., UK.

Panacur 4 % powder: fenbendazole 4 %, manufactured by Intervet UK Ltd.

Whipsnade Grazer: manufactured by Clarke & Butcher, Soham, Cambs., UK.

Zoo Grazer G.P. Diet: manufactured by SDS/Mazuri, Witham, Essex, UK.
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