MINERAL DEFICIENCIES IN TULE ELK, OWENS VALLEY, CALIFORNIA

Authors: Johnson, Heather E., Bleich, Vernon C., and Krausman, Paul R.

Source: Journal of Wildlife Diseases, 43(1) : 61-74

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-43.1.61

Journal of Wildlife Diseases, 43(1), 2007, pp. 61–74

MINERAL DEFICIENCIES IN TULE ELK, OWENS VALLEY, CALIFORNIA

Heather E. Johnson,[1,3] Vernon C. Bleich,[1] and Paul R. Krausman[2]

[1] Sierra Nevada Bighorn Sheep Recovery Program, California Department of Fish and Game, 407 West Line Street, Bishop, CA 93514, USA
[2] School of Renewable Natural Resources, The University of Arizona, Tucson, AZ 85721, USA
[3] Corresponding author (email: hjohnson@dfg.ca.gov)

ABSTRACT: Male tule elk (*Cervus elaphus nannodes*) are susceptible to high rates of antler breakage in Owens Valley, California. We hypothesized that a mineral deficiency in the diet predisposed male elk to antler breakage. We analyzed elk antler, liver, and forage samples to identify mineral imbalances. We compared the mineral content of livers and antlers from elk in Owens Valley to samples taken from tule elk at Grizzly Island Wildlife Area, a population experiencing normal rates (<5%) of antler breakage. Antler and liver samples were collected from 1989 to 1993, and in 2002, and were tested for calcium (Ca), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), molybdenum (Mo), phosphorus (P), sulfur (S), and zinc (Zn). Mineral levels from antler and liver samples were compared to reference values established for elk and deer. We also compared the mineral content of elk forage in Owens Valley, collected in 2002–03, to dietary reference values established for cattle. In antlers, Ca, Fe, and Mg levels were higher in Owens Valley elk than in Grizzly Island elk, although all mineral levels were lower than reference values established for deer antlers. In liver samples, Cu levels from elk in Owens Valley were lower than those from Grizzly Island and lower than minimum reference values; liver Ca and Mo levels were higher in elk from Owens Valley than in those from Grizzly Island. Compared to reference values, elk forage in Owens Valley had high levels of Ca and Mo, and low levels of Cu, P, and Zn. Mineral analyses from antlers, livers, and forage suggest that tule elk in the Owens Valley are Cu and/or P deficient. High levels of Mo and Ca may exacerbate Cu and P deficiencies, respectively. Bone fragility is a symptom of both deficiencies, and an imbalance in Cu, P, or a combination of both, may predispose male tule elk in the Owens Valley to antler breakage.

Key words: Antler breakage, California, *Cervus elaphus nannodes*, copper, mineral deficiency, Owens Valley, phosphorus, tule elk.

INTRODUCTION

An abnormally high rate of antler breakage has occurred in the tule elk (*Cervus elaphus nannodes*) population inhabiting the Owens Valley, California (McCullough, 1969). In 2002 and 2003, 82% of the males harvested in the Owens Valley had broken antler tines and 36% had broken main beams (Johnson et al., 2005). This high rate of antler breakage has not been described in any other cervid population; estimates for rates of antler breakage typically are <5% (Henshaw, 1971).

Antler development reflects the body condition and nutritional status of male cervids (Severinghaus et al., 1950; French, 1956; Cowen and Long, 1962; Hyvarinen et al., 1977). Because antler growth requires protein, nutrients, and energy, and these resources are allocated to antler development only after requirements for body growth and maintenance have been met (Goss, 1983), antler characteristics have been used to assess the health and habitat condition of elk populations (McCorquodale et al., 1989). Although elk antlers in the Owens Valley develop normally with respect to size and shape, they easily break when males participate in sparring matches and fights (McCullough, 1969; Johnson et al., 2005). The brittleness of the antlers is characteristic of a problem with the structure or composition of the bone (Martin, 1991). Although overall forage quality is important for antler development, bone fragility has been specifically associated with mineral imbalances (French, 1956; Underwood, 1977; Gogan et al., 1988; Bleich, 1990; Robbins, 1993).

Minerals related to the growth and development of antlers and bones are calcium (Ca), copper (Cu), and phosphorus (P) (Robbins, 1993; Puls, 1994). In cervids, deficiencies of these minerals can reduce antler growth and strength, and result in bone abnormalities, bone fractures, poor bone mineralization, osteoporosis, and rickets (French, 1956; Franzmann et al., 1975; Gogan et al., 1988; Puls, 1994). When Ca, Cu, or P is not sufficient in an animal's diet, a primary (or simple) deficiency is produced. A secondary (or complex) deficiency occurs when Ca, Cu, or P is sufficient in the diet, but cannot be properly absorbed. Imbalances in levels of iron (Fe), magnesium (Mg), manganese (Mn), molybdenum (Mo), sulfur (S), and zinc (Zn) can limit absorption of Ca, Cu, and P (Underwood, 1977). Unfortunately, however, little is known about the mineral requirements of wildlife species, or signs associated with mineral imbalances. Information on wildlife nutrition has been based primarily on research from domestic animals (Robbins, 1993), and is not necessarily applicable to the requirements of free-ranging wildlife (Robbins et al., 1985; Samson et al., 1989).

Explanations for antler breakage in Owens Valley tule elk have been proposed, but no cause has yet been conclusively determined. McCullough (1969) originally attributed antler breakage to low levels of dietary P. Although P was low in Owens Valley plants, P in elk antler samples was comparable to samples collected from healthy herds in other parts of the state (McCullough, 1969). More recently, Cu deficiency has been suspected as a factor in antler breakage. Indeed, cattle ranchers in Owens Valley routinely treat livestock with supplements to prevent symptoms of Cu deficiency. Although Cu deficiency has occurred in the tule elk population at Point Reyes National Seashore, California, the clinical signs differed from those observed in Owens Valley. Elk from Point Reyes developed clubbed, malformed antlers (Gogan et al., 1988), but did not possess broken antlers. Additionally, elk from Point Reyes exhibited other signs characteristic of Cu deficiency, including loss of hair pigment, brittle pelage, lack of thriftiness, and a stilted gait (Gogan et al., 1988, 1989), symptoms not observed among elk in Owens Valley.

Because antler development reflects the nutritional condition of an animal, it is important to understand the factors contributing to antler breakage. We hypothesized that antler breakage was the consequence of a mineral imbalance and, therefore, quantified the mineral content of elk antler, liver, and forage samples to comprehensively examine the mineral nutrition of Owens Valley tule elk. Mineral levels in livers and antlers were compared to samples from tule elk at Grizzly Island Wildlife Area, a population where <5% of the males exhibit broken antlers after the rut (Bleich, unpubl. data).

MATERIALS AND METHODS

Study area

The Owens Valley is located in Inyo County, California (39°59′N, 118°13′W). The valley is oriented in a north–south direction, and is approximately 195 km long and 26 km wide, ranging in elevation from 1,225 m in the north to 1,160 m in the south (Bleich et al., 2001). The Sierra Nevada is on the western side of the Owens Valley and the White and Inyo mountains are on the eastern side; these ranges reach elevations of 4,200 m. The valley lies in the rain shadow of the Sierra Nevada, and receives approximately 13 cm of rainfall per year (National Oceanic and Atmospheric Administration, 2003). Winter temperatures frequently fall below freezing, and summer temperatures often are >37 C.

Vegetation in the Owens Valley consists of Great Basin and Mohave Desert shrub communities (McCullough, 1969). Saltbush (*Atriplex* spp.), rabbitbrush (*Chrysothamnus nauseosum*), and sagebrush (*Artemisia* spp.) dominate the uplands, and greasewood (*Sarcobatus vermiculatus*), saltgrass (*Distichlis spicata*), and shadescale (*Atriplex confertifolia*) dominate the lowlands (Bleich et al., 2001). The Owens River flows south through the valley creating a riparian area composed largely of willow (*Salix* spp.) and cottonwood

(*Populus fremontii*) forests, and cattail (*Typha domingensis*) marshes. Cattle grazing occurs throughout the valley, but agriculture is limited to a few alfalfa fields. Tule elk inhabit the valley floor, which is owned by the Los Angeles Department of Water and Power, the Bureau of Land Management, and the US Forest Service (McCullough, 1969).

Grizzly Island Wildlife Area is located approximately 65 km northeast of San Francisco, in Solano County, California (38°08′N, 121°58′W). Grizzly Island encompasses roughly 3,450 ha of the Sacramento River delta flatland, is bordered on the south by the confluence of the Sacramento and San Joaquin rivers, and is surrounded by a series of sloughs and canals. The elevation at Grizzly Island ranges from <2 m above to 1 m below sea level, and the area receives approximately 60 cm of rainfall per year (Gogan and Barrett, 1987). Native vegetation on the island is dominated by common tule (*Scirpus acutus*), cattail (*Typha latifolia*), rushes (*Juncus* spp.) and sedges (*Carex obnupta* and *Carex senta*) (Kuchler, 1977). Grizzly Island is classified as a tule marsh, but many of the native plants have been replaced with cultivated grain to support waterfowl during winter. Grizzly Island is managed by the California Department of Fish and Game (CDFG), and there is no cattle grazing in the area.

Antler and liver analyses

We operated hunter check-stations to collect tule elk antler and liver samples. Check-stations were operated during August and November in Owens Valley, and August and September at Grizzly Island. Tule elk are unique among elk subspecies in the timing of antler development and mineralization. In tule elk, the antler mineralization process takes place in late June and July, and the elk shed their velvet by the end of July or early August (Johnson et al., 2005). Although liver minerals can be depleted during antler mineralization, mineral stores should have been replenished before hunter check-station samples were collected. Samples were collected from 1989 to 1993 and in 2002 in Owens Valley, and in 1992, 1993, and 2002 at Grizzly Island.

To evaluate causes of antler breakage we first examined the mineral composition of hardened antler material. We collected an antler sample from harvested males by drilling a hole (0.635-cm drill bit) approximately 2 cm above the pedicle into the center of the antler. We drilled to a depth of 3 cm and collected the antler shavings for analysis. We carefully cleaned each antler prior to drilling to ensure no contamination from surface materials. We also diagrammed the morphology of each antler, and recorded the number of intact tines, broken tines, and broken main beams.

Additionally, we analyzed the mineral content of tule elk liver samples. The liver serves as a storage bank for many minerals and is an important indicator of chronic mineral deficiencies and toxicities (Paynter, 1987; Grace and Wilson, 2002). At check-stations we collected liver material (200 g) from harvested males and froze samples at −20 C until chemical analysis.

Antler samples collected from 1989 to 1993 were ashed and analyzed for Ca and P. Calcium levels were determined by flame atomic absorption spectrophotometry (AAS; Association of Official Analytical Chemists, 1990 [968.08]), and P levels were determined by colorimetric spectrophotometry (Association of Official Analytical Chemists, 1990 [965.17]). Calcium and P, analyzed at the Manna-Pro Corporation Laboratory in Fresno, California, USA, were reported as parts per million (ppm) dry weight. For increased diagnostic power, antler samples collected in 2002 were tested for Ca, Cu, Fe, Mg, Mn, Mo, P, S, and Zn. Samples were analyzed at the Michigan State University Diagnostic Center for Population and Animal Health (DCPAH) by radial flow inductively coupled argon plasma emission spectroscopy (ICP; Stowe et al., 1985), and reported as ppm dry weight.

Liver samples collected from 1989 to 1993 were analyzed for levels of Cu and Mo. Mineral levels were analyzed by flame AAS, and were reported as ppm wet weight (Table 1; California Department of Fish and Game, 1993). Analyses were conducted by the CDFG Water Pollution Control Laboratory, Rancho Cordova, California, USA. To increase diagnostic power, liver samples collected in 2002 were analyzed for Ca, Fe, Mg, Mn, S, P, and Zn, in addition to Cu and Mo. Mineral levels from samples collected in 2002 were analyzed by ICP (Stowe et al., 1985), and reported as ppm wet weight; samples were analyzed at DCPAH.

Because mineral levels are interrelated, we used multivariate analysis of variance (MANOVA) simultaneously to compare antler minerals, and then liver minerals, between Owens Valley and Grizzly Island. For both antler and liver samples we also conducted univariate comparisons of mineral levels among the two populations using the adjusted F statistic to account for correlations among mineral level responses. Because antler samples and liver samples collected from 1989 to 1993 were analyzed for different minerals than samples

TABLE 1. Average macromineral and trace mineral levels (parts per million [ppm]±SE) in male tule elk antler and liver samples from Owens Valley and Grizzly Island, California, 1989–2002, and reference values (Puls, 1994).

	Antler					Liver				
Minerals	*n*	Owens Valley	*n*	Grizzly Island	Reference levels for deer	*n*	OV	*n*	GI	Reference levels for elk
Calcium	70	207,132±16,491	39	195,026±13,190	230,000–240,000	16	49.68±2.19	8	39.05±3.10	35–65
Copper	15	0.47±0.1412[a]	7	0.69±0.17	6.3–6.5	77	8.67±1.35	43	47.99±1.80	20–120
Iron	15	20.78±15.75	7	54.63±25.05	Unavailable	16	151.31±21.47	8	132.25±29.40	100–200
Magnesium	15	4,217±2723	7	3,291±247	5,585–5,690	16	159.88±3.44	8	161.13±4.72	110–210
Manganese	15	2.87±4.132[b]	7	4.09±3.74	22–34	16	2.14±0.21	8	2.11±0.29	2.0–6.0
Molybdenum	15	Undetected[c]	7	Undetected	Unavailable	77	1.13±0.04	43	0.82±0.05	0.14–1.40[d]
Phosphorus	70	89,678±5,299	39	90,210±6,626	104,000–106,000	16	3,788.31±97.27	8	3,571.25±133.19	2,000–4,000[d]
Sulfur	15	1,127±229	7	1,072±173	2,255–2,276	16	2,183.33±61.62	8	2,043.75±84.38	2,550–2,678
Zinc	15	66.70±10.64	7	61.14±5.56	Unavailable	16	20.40±1.77	8	19.24±2.42	23–80
Calcium:phosphorus	15	2.13±0.05	7	2.14±0.02	2.1–2.2		—		—	—

[a] Copper levels only detectable in 8 of 15 samples (detection limit=0.250 ppm).

[b] Manganese levels only detectable in 10 of 15 samples (detection limit=0.250 ppm).

[c] Molybdenum detection limit was 1.00 ppm.

[d] Reference values for cattle.

collected in 2002, we ran separate antler and liver multivariate and univariate analyses for each sampling period. Antler Cu and Mn were excluded in the 2002 MANOVA because they were undetected in several samples (detection limit=0.250 ppm). Antler Mo was not detected in any 2002 sample and, thus, also was excluded from the antler analysis (detection limit=1.00 ppm). We log-transformed antler mineral levels as necessary to meet assumptions of normality. Additionally, we used Student's *t*-test to compare the Ca:P ratio in antler samples between study areas. Because Ca and P were measured in all antler samples, the Ca:P ratio was evaluated using samples from all years combined.

We compared antler and liver mineral levels from Owens Valley and Grizzly Island to reference values for ruminant nutrition (Table 1). Average levels of Ca, Cu, Mg, Mn, P, and S in elk antlers were compared to reference values available for deer antlers (Puls, 1994). Average liver levels of Ca, Cu, Fe, Mg, Mn, S, and Zn were compared to reference values for elk (Puls, 1994). Because elk reference values were not available for liver Mo and P, we compared averages from these mineral levels to reference values for cattle (Puls, 1994).

From males harvested in November in Owens Valley, after the rut, we used linear regression to determine whether the percentage of antler breakage was associated with the antler Ca:P ratio or liver Cu. We calculated the percentage of antler breakage as the total number of broken tines per total number of tines prior to breakage. If an antler, or part of an antler, was missing because of a break in a main beam, we assumed that the total number of tines on the broken antler was equal to the total number of tines on the intact antler. No harvested males included in the analysis had both main beams broken. We used the antler Ca:P ratio because bone provides more reliable Ca and P information than liver (McDowell, 1992; Puls, 1994). Similarly, we used liver Cu because the liver is a better indicator of Cu status than antler or bone (Paynter, 1987; Puls, 1994). Interference of Fe, Mg, Mn, Mo, S, or Zn would be reflected in bone Ca and P, and in liver Cu. We used a log transformation on percentage of antler breakage and Cu levels to more closely approximate normal distributions.

Forage analyses

To describe the general mineral content of elk forage in Owens Valley, we collected plant samples seasonally in autumn (October 2002), winter (January 2003), spring (April 2003), and summer (July 2003). Because male elk do not consume as much forage during the rut (Bobek et al., 1990), we timed the collection of seasonal forage samples to avoid rutting activity, which occurs in the Owens Valley between mid-August and mid-September (Johnson et al., 2005). Each season we collected eight forage species (Table 2) that accounted for a majority of the diet during that time of year (Logsdon, 1973; Curtis, unpubl. data). For each species, ≥20 plants were sampled randomly throughout the study area in the foraging manner of elk (Haufler and Servello, 1996). We collected equal amounts of each plant, obtaining a total of 500 g of plant material that was compiled in a single sample for each forage species. We stored forage samples in brown paper bags until they could be oven-dried for 72 hr at 22 C. We ground forage samples through a 1-mm screen in a Model 4 Thomas-Wiley laboratory mill (Thomas Scientific, Swedesboro, New Jersey, USA).

Each forage sample was examined for levels of Ca, Cu, Fe, Mg, Mn, Mo, P, S, and Zn by axial-view ICP (Association of Official Analytical Chemists, 2000 [985.01]) at Cumberland Valley Analytical Services in Maugansville, Maryland, USA (Table 2). Macrominerals (Ca, Mg, P, and S) were reported as percentage of dry weight, and trace minerals (Cu, Fe, Mn, Mo, and Zn) were reported as ppm dry weight. We also determined the ratios of Ca:P and Cu:Mo, both of which are important in detecting mineral imbalances (McDowell, 1992; Puls, 1994). For each season, we calculated mean mineral levels from the eight forage species sampled. Seasonal averages were then compared to values recommended for ruminant nutrition. Because specific reference values have not been identified for free-ranging elk, we referenced mineral levels recommended for domestic cattle (Puls, 1994).

RESULTS

Antler and liver analyses

We collected and analyzed 70 antler samples from tule elk in Owens Valley and 39 antler samples from tule elk in Grizzly Island. In Owens Valley, we analyzed 55 antler samples collected from 1989 to 1993, and 15 samples from 2002. In Grizzly Island, we analyzed 32 samples collected from 1992 to 1993, and seven in 2002.

Antler minerals in samples collected

TABLE 2. Mineral levels in seasonal tule elk forage, Owens Valley, California, 2002–03. Macrominerals are reported as percent content dry weight, and trace minerals are reported as parts per million (ppm) dry weight.

Plant species	Calcium %	Magnesium %	Phosphorus %	Sulfur %	Copper ppm
October 2002					
Bassia hyssopifolia	0.40	0.28	0.31	0.29	10.44
Atriplex canescens	1.31	0.87	0.12	0.45	10.59
Sarcobatus vermiculatus	0.99	0.35	0.10	0.45	10.88
Juncus spp.	0.65	0.20	0.08	0.41	5.12
Glycyrrhiza lepidota	2.39	0.39	0.12	0.35	10.83
Stipa speciosa	0.29	0.07	0.04	0.10	3.37
Salix spp.	2.07	0.48	0.12	0.62	6.89
Melilotus alba	1.35	0.32	0.22	0.29	9.59
January 2003					
Atriplex polycarpa	1.59	0.90	0.15	0.45	12.32
Artemisia tridentata	0.87	0.21	0.21	0.29	12.59
Atriplex canescens	1.57	0.91	0.16	0.45	11.49
Sarcobatus vermiculatus	2.10	0.45	0.05	0.13	9.91
Stipa speciosa	0.36	0.04	0.03	0.06	4.40
Sporobolus airoides	0.39	0.10	0.05	0.11	3.49
Atriplex confertifolia	2.42	0.80	0.10	0.64	7.28
Eurotia lanta	1.53	0.47	0.18	0.22	7.17
April 2003					
Xylorhiza tortifolia	0.77	0.26	0.24	0.15	10.50
Artemisia spinescens	1.18	0.43	0.21	0.40	10.76
Sarcobatus vermiculatus	0.70	0.23	0.23	0.36	14.36
Psorothamnus arborescens	1.80	0.30	0.24	0.24	4.50
Stipa speciosa	0.29	0.09	0.14	0.18	3.12
Sporobolus airoides	0.28	0.17	0.15	0.27	3.36
Salix spp.	1.41	0.37	0.29	0.61	9.21
Grayia spinosa	1.79	1.10	0.20	0.62	5.74
July 2003					
Bassia hyssopifolia	0.52	0.29	0.29	0.54	9.84
Sarcobatus vermiculatus	0.73	0.21	0.11	0.43	8.37
Psorothamnus arborescens	3.52	0.42	0.11	0.29	5.50
Juncus spp.	0.37	0.13	0.14	0.30	5.72
Glycyrrhiza lepidota	0.62	0.24	0.25	0.30	7.98
Sporobolus airoides	0.35	0.20	0.19	0.37	3.61
Salix spp.	0.45	0.19	0.19	0.32	5.77
Melilotus alba	1.44	0.41	0.26	0.44	9.30

from 1989 to 1993 differed between elk populations (MANOVA, $F_{2,85}=13.68$, $P<0.001$). Calcium was higher in Owens Valley antler samples than in Grizzly Island samples ($F_{1,86}=7.85$, $P=0.006$), whereas P was lower in Owens Valley antler samples in than Grizzly Island samples ($F_{1,86}=7.85$, $P=0.006$).

In 2002, antler samples were analyzed for Fe, Mg, S, and Zn, in addition to Ca and P. Antler mineral levels differed between the elk populations (MANOVA, $F_{6,15}=12.51$, $P<0.001$). Owens Valley antler samples were higher in Ca ($F_{1,20}=12.58$, $P=0.002$) and Mg ($F_{1,20}=58.26$, $P<0.001$) and lower in Fe ($F_{1,20}=3.64$, $P=0.071$) and P ($F_{1,20}=18.17$, $P<0.001$) than were Grizzly Island samples. Populations did not differ in antler mineral levels of S or Zn (both tests, $P>0.26$). Copper was detected in only eight of 15 samples from Owens Valley, and in six of seven samples from Grizzly Island. Magnesium was detected in all samples from Grizzly Island, but in only 10 of 15

TABLE 2. Extended.

Iron ppm	Manganese ppm	Molybdenum ppm	Zinc ppm	Calcium:phosphorus %	Copper:molybdenum ppm
126.62	39.73	4.31	21.94	1.33	2.58
617.17	74.39	3.19	15.73	11.40	3.32
335.61	116.52	6.54	10.13	9.71	2.14
82.13	218.53	9.54	7.15	7.66	0.72
143.01	48.92	9.43	7.73	19.95	1.31
395.21	42.92	1.67	5.53	10.15	2.36
139.68	260.50	2.06	55.14	17.98	10.24
113.84	26.94	33.09	14.69	6.19	0.31
375.66	218.10	2.50	31.08	10.40	5.40
542.10	63.37	2.99	25.19	4.13	4.76
302.20	92.61	3.27	20.61	9.79	3.63
1,539.90	187.63	2.13	22.82	42.21	2.31
375.34	28.83	1.29	6.63	15.46	3.88
294.89	20.02	3.47	4.54	7.75	1.61
562.58	261.16	6.03	23.65	23.99	7.55
845.70	105.55	1.13	14.49	8.99	7.17
150.07	33.73	2.47	16.57	3.17	12.18
622.94	99.92	3.03	8.76	5.69	3.65
126.46	144.88	1.73	25.53	3.10	9.40
180.57	33.86	2.43	13.31	7.54	2.17
120.93	39.95	1.75	10.61	2.16	1.87
186.05	42.90	4.75	4.58	1.91	0.94
129.18	128.95	0.80	169.74	4.86	12.33
283.46	106.97	3.20	11.66	9.30	1.83
122.54	56.86	7.25	26.31	1.78	1.65
153.38	65.90	5.40	16.29	6.27	1.67
276.14	57.57	2.59	15.55	32.45	2.23
69.57	127.11	7.09	22.52	2.64	0.94
118.32	34.08	1.52	38.81	2.50	5.23
213.71	24.94	8.43	11.31	1.88	0.45
133.87	62.04	5.68	24.21	2.34	2.21
105.75	36.85	43.15	25.57	5.45	0.23

samples from Owens Valley. The Ca:P ratio, calculated for antler samples collected during all years, was higher in Owens Valley samples than in Grizzly Island samples ($t_{108}=-5.33$, $P<0.001$). Average antler mineral levels from Owens Valley and Grizzly Island samples all were below reference values for deer (Table 1).

We collected 77 liver samples from male tule elk in Owens Valley and 43 liver samples from male tule elk in Grizzly Island. From 1989 to 1993, 61 liver samples were collected and analyzed from Owens Valley, and 35 from Grizzly Island. In 2002, an additional 16 liver samples were collected and analyzed from Owens Valley, and eight from Grizzly Island.

Liver minerals sampled from 1989 to 1993 differed between elk populations (MANOVA, $F_{2,94}=132.59$, $P<0.001$). Liver Cu levels were lower in Owens Valley elk than in Grizzly Island elk ($F_{1,95}=222.19$, $P<0.001$), whereas liver Mo levels were higher in Owens Valley

elk than in Grizzly Island elk ($F_{1,95}=22.65$, $P<0.001$).

Overall, mineral levels for liver samples collected in 2002 were also different between elk populations (MANOVA, $F_{9,12}=5.60$, $P=0.002$). Copper was lower in Owens Valley liver samples than in Grizzly Island samples ($F_{1,22}=27.48$, $P<0.001$), whereas Ca was higher in Owens Valley liver samples than in Grizzly Island samples ($F_{1,22}=7.82$, $P=0.0105$). Liver Mo was also higher in Owens Valley samples than in Grizzly Island samples ($F_{1,22}=5.18$, $P=0.033$). No difference existed between Owens Valley and Grizzly Island elk samples in levels of liver Fe, Mg, Mn, Mo, P, S, or Zn (all tests $P\geq0.20$).

Although some liver minerals differed between the populations, means of most mineral levels were within reference values (Table 1). Average values for liver Ca, Mg, Mn, Mo, and P all were adequate for both populations. Although the means were considered adequate for Fe, a few elk from Owens Valley had liver concentrations below the recommended value, and a few elk in both populations had liver Mn levels below the recommended value. Approximately 16% of the samples from Owens Valley and 10% of the samples from Grizzly Island had Mo liver levels above those recommended for cattle. Liver Cu levels in Grizzly Island elk were within recommended ranges, but only 10% of the elk from Owens Valley had liver Cu above the minimum threshold recommended for elk. Phosphorus liver levels were at the upper end of the recommended range for both Owens Valley and Grizzly Island elk, whereas mean S and Zn values in both populations were slightly below levels recommended for adequate nutrition.

Antler mineral composition and antler breakage data were collected at hunter check-stations from 36 males in Owens Valley during 1989–1993 and in 2002. Percentage of antler breakage in elk was not associated with the antler Ca:P ratio ($r^2=0.003$, $P=0.73$), or liver Cu ($r^2=0.07$, $P=0.13$).

Forage samples

Mineral levels in elk forage indicated seasonal deficiencies and toxicities (Table 2, 3). In all seasons, mean forage Ca was above the level recommended for adequate nutrition, but below toxic levels (Table 3). Of the 32 plant samples tested for Ca, 12 contained levels of dietary Ca considered to be toxic (Puls, 1994). Conversely, mean Cu values in elk forage were below recommended levels, but above values considered to indicate deficiency. Of the 32 forage species tested during the year, 12 were deficient in Cu, a few in each season. Dietary Fe was adequate during autumn, spring, and summer. Winter Fe was higher than recommended, but below toxic levels. Magnesium in elk forage exceeded, or nearly exceeded, recommended values in autumn, winter, and spring, but did not reach toxic levels; summer plants were considered adequate in Mg. Levels of dietary Mn were adequate for ruminant nutrition in all seasons. Molybdenum was considered adequate in winter and spring plants, but the mean level in autumn plants reached nearly toxic levels and Mo levels were considered toxic in summer plants. Levels of S in forage plants were at the high end of the reference range for autumn, spring, and summer, but S in winter plants was adequate. For all seasons, average Zn levels were below recommended values, but were not deficient. Seven of 32 forage species were considered deficient in Zn. The mean Ca:P ratio was toxic in autumn and winter plants, with 11 of 16 forage samples exceeding toxic Ca:P ratios. Conversely, the Cu:Mo ratio was abnormally low in 13 of 16 forage species collected in autumn and summer. Even during winter and spring, Cu:Mo ratios in six of 16 species of plants were below recommended values.

TABLE 3. Average macromineral and trace mineral levels (±SD), calcium:phosphorus ratios (±SD), and copper:molybdenum ratios (±SD) of tule elk forage sampled seasonally in Owens Valley, California, 2002–03, and reference values for domestic cattle (Puls, 1994). Average mineral levels were calculated from the eight plant species most common in the diet during each season. Macrominerals are reported as percent content dry weight, and trace minerals are reported as parts per million (ppm) dry weight.

Minerals	October	January	April	July	Reference values for cattle		
					Deficient	Adequate	Excessive/toxic
Calcium (%)	1.18±0.76	1.35±0.75	1.03±0.08	1.00±0.13	<0.26	0.38–0.81	1.4–?
Magnesium (%)	0.37±0.24	0.49±0.35	0.37±0.04	0.26±0.01	0.03–0.2	0.25–0.35	1.0–4.0
Phosphorus (%)	0.14±0.09[a]	0.12±0.07[a]	0.21±0.01[a]	0.19±0.07[a]	<0.3	0.35–0.45	>1.0
Sulfur (%)	0.37±0.15	0.29±0.20	0.35±0.02	0.37±0.01	Unavailable	0.21–0.36	>0.5
Copper (ppm)	8.46±2.95	8.58±3.53	7.69±0.51	7.10±0.27	<6.0	10–25	>100
Iron (ppm)	244±188	605±419	225±21	149±8	<40	100–500	>4,000
Manganese (ppm)	104±89	122±90	79±6	58±4	1	40–200	2,000–4,000
Molybdenum (ppm)	8.73±10.31	2.85±1.55	2.52±0.15	10.14±1.69[b]	Unavailable	0.5–3.5	10–203
Zinc (ppm)	17.26±16.25	18.63±9.29	32.59±6.97	22.57±1.06	2–10	50–100	>5,000
Calcium:phosphorus (%)	10.55±6.07[b]	15.35±12.40[b]	4.72±0.33	4.72±0.33	<1.1	1.5–7:1	>8.1
Copper:molybdenum (ppm)	2.87±3.14[a]	4.54±2.12	5.55±0.61	1.83±0.20[a]	<3:1	4.3:1	6–10:1

[a] Indicates deficient mineral levels.

[b] Indicates toxic mineral levels.

DISCUSSION

The complexities associated with mineral nutrition and metabolism can confound the diagnosis of a mineral-related disease. The manifestations of a mineral deficiency can differ among animals of different ages, gender, and species, and can also be affected by the severity of the deficiency and the length of time the deficiency has occurred (Underwood, 1977; Paynter, 1987; Puls, 1994). Although clinical signs usually indicate the presence of a mineral imbalance, symptoms are rarely disease-specific; a single disorder can be symptomatic of several different imbalances (Underwood and Suttle, 1999). As a result, the diagnosis of a mineral-related disease is usually accomplished through a combination of clinical and biochemical examinations (Underwood and Suttle, 1999). For a complete understanding of the mineral nutrition of tule elk in Owens Valley, we used a combination of antler, liver, and forage analyses to examine possible mineral deficiencies and toxicities. Results from all analyses suggest that antler breakage in Owens Valley elk is related to a deficiency in Cu, P, or both.

Liver Cu levels in Owens Valley tule elk were similar to those in other ruminant populations experiencing Cu deficiency. On average, elk from Owens Valley had 8.7 ppm liver Cu, much lower than the 55.2 ppm liver Cu found in Grizzly Island elk and lower than elk reference values, which ranged from 20 to 120 ppm (Puls, 1994). Tule elk from the Point Reyes population, also diagnosed as Cu deficient, had <10 ppm liver Cu (Gogan et al., 1989). Populations of red deer (*Cervus elaphus*) have been afflicted with enzootic ataxia and osteochondrosis, both of which are symptomatic of Cu deficiency, when liver Cu was <20 ppm (Barlow, 1978; Audigé et al., 1995). Cattle have had spontaneous bone fractures, similar to antler breakage in the Owens Valley, after grazing on Cu-deficient pastures (Cunningham, 1950). Although cattle are recommended to have >25 ppm liver Cu (Puls, 1994), individuals with bone fractures had levels of liver Cu <10 ppm. An analysis of forage minerals in Owens Valley indicated that Cu levels were low in plants. In all seasons, mean Cu values in forage were below recommended values, but above values considered deficient.

Although dietary Cu was low, it is unclear whether elk are experiencing a primary deficiency from insufficient forage Cu, or a secondary deficiency in which low dietary Cu is exacerbated by high dietary Mo. Molybdenum is an antagonist of Cu, and high levels of Mo reduce the Cu available to the body (Underwood, 1977). The interaction between Cu and Mo is dependent upon high levels of S. In the rumen, sulfate is reduced to sulfide, and sulfide and Mo react to form thiomolybdates. In turn, thiomolybdates have a strong affinity for Cu, forming insoluble Cu thiomolybdate that is unavailable for absorption into the body (Suttle 1975). Because bacteria in the rumen generate large amounts of sulfide, ruminants are particularly susceptible to Cu:Mo imbalances. The alkaline soils in Owens Valley are characteristically high in Mo, causing McCullough (1969) to originally suspect that tule elk were suffering from molybdenosis, a Mo-induced Cu deficiency (Robbins 1993). Although Mo was high in several forage species McCullough tested, liver Cu:Mo ratios ($n=5$) were favorable, and he concluded that molybdenosis was not implicated in antler breakage. Liver Mo was relatively high in our samples compared with liver Cu. Additionally, Mo in our forage samples reached toxic levels during autumn and summer, and was accompanied by high levels of dietary S, a precursor to secondary Cu deficiency. Through processes of mineral metabolism, the combination of low dietary Cu and high dietary Mo likely induced a Cu deficiency in tule elk in Owens Valley.

Phosphorus deficiency, one of the most

common deficiencies in grazing ruminants (McDowell, 1985), also may be occurring among tule elk in Owens Valley. Either low dietary P or an extreme imbalance of Ca and P can yield symptoms of P deficiency (McDowell, 1992). In Owens Valley, liver and antler samples had higher levels of Ca, and lower P levels, than did samples from Grizzly Island. Additionally, forage plants in Owens Valley exceeded adequate Ca levels and were deficient in P in all seasons. For mineral nutrition, the absolute amounts of a mineral often are not as important as the relative proportions of the minerals that interact with one another (McDowell, 1992). High Ca and low P resulted in a Ca:P ratio considered toxic in forage during autumn and winter (Puls, 1994). The combination of low dietary P and high dietary Ca could cause tule elk to be deficient in P.

Nutritional requirements are not well understood for most species of wildlife, which was reflected in the mineral reference levels to which we compared elk samples. Because mineral reference ranges have not been identified for elk antlers or forage, we compared our samples to ranges recommended for deer and cattle, respectively. Reference values for cattle should be used with caution, because nutritional requirements of domestic cattle may not adequately represent the needs of free-ranging elk. Even the reference values for deer antlers were not comparable to elk antlers from Owens Valley or Grizzly Island, because all minerals we tested were present in levels that were lower than reference levels for deer. As long as mineral requirements are undetermined for wildlife, detection and diagnosis of mineral-related diseases will be difficult.

Although mineral analysis of foodstuffs can be used to identify whether a mineral deficiency is induced from primary or secondary means (Paynter, 1987; Underwood and Suttle, 1999), the interpretation of mineral levels in our forage samples should be regarded with caution. Because we could not assess the exact diets of free-ranging tule elk, we analyzed the primary plant species consumed during each season. We weighted all plants equally in the calculation of average seasonal mineral levels; however, because animals consume plants in different amounts, considerable individual variation in the mineral quantities ingested would be expected. Therefore, our forage analysis identified mineral levels available to elk, not levels necessarily ingested by elk. Furthermore, seasonal averages of several minerals had large standard deviations. Even within a season, individual plants can yield dramatically different mineral levels as a function of soil conditions, age, developmental stage, and species (Delhaize et al., 1987). For example, among plants collected in spring, Cu varied from 3.12 ppm to 14.36 ppm (Table 2). Depending on the diet consumed by elk, dietary Cu could be adequate or, alternatively, severely deficient.

Severity of antler breakage was not associated with the Ca:P ratio or liver Cu status of male tule elk in Owens Valley. Because virtually all males were deficient in liver Cu, antler fragility may not be related to small changes in Cu below a certain threshold. Similarly, all males with Ca:P ratios exceeding a certain level may be equally susceptible to breaking antlers. Severity of antler breakage may be a function of behavior, rather than minute differences in mineral levels (Johnson et al., 2005). For example, if antler fragility is characteristic of all male elk in Owens Valley, individuals participating in more aggressive behaviors involving antler contact would have a greater probability of breaking tines than males participating in fewer behaviors involving antler contact.

Because bone disorders are characteristic of Cu and P deficiency, it is difficult to determine whether antler breakage is the consequence of one or the other of the imbalances, or a combination of the two. In addition to having broken antlers, McCullough (1969) reported that the tule

elk population in Owens Valley had a high number of crippled animals in the population. Additionally, skeletons of tule elk found in Owens Valley showed evidence of broken and mended bones (McCullough, 1969). Ruminants deficient in Cu have exhibited fragile long bones, spontaneous bone fractures, osteoporosis, and poor bone mineralization (Cunningham, 1950; Davis, 1950; Suttle and Angus, 1978; Underwood and Suttle, 1999). Similarly, ruminants deficient in P have demonstrated spontaneous bone fractures, osteoporosis, and poor bone mineralization (Shupe et al., 1988; McDowell, 1992; Puls, 1994). Although Cu deficiency reduces the cross-linkages in bone collagen (Rucker et al., 1969, 1975), P deficiency causes a decrease in the mineral content of the organic matrices of bone (McDowell, 1992). Either Cu or P deficiency could be responsible for inducing antler fragility.

Because bone fragility is characteristic of both Cu and P imbalances, and elk in Owens Valley do not exhibit other obvious clinical signs related to these deficiencies, we cannot definitively determine the cause of antler breakage. A mineral deficiency is disconcerting because minerals are essential for numerous metabolic processes in the body, and are required for proper growth, development, and reproduction (Underwood, 1977; McDowell, 1992). If a mineral deficiency is inhibiting normal antler development in tule elk, other physiological processes may be affected. Recently, low recruitment rates in Owens Valley tule elk have raised concern over the potential influence of mineral deficiencies on elk reproduction. We propose that a mineral supplementation study be conducted on tule elk in Owens Valley to explicitly determine the cause of antler breakage. Throughout antler development, males should be treated for Cu deficiency, P deficiency, or both and monitored for rates of antler breakage. Mineral supplementation will allow managers to confirm the cause of antler breakage, and potentially identify other problems affecting herd health.

Antler breakage among tule elk in Owens Valley likely may be a consequence of introducing a species into an area in which it had not historically occurred. Tule elk are not native to the Owens Valley, being translocated in 1933 (McCullough, 1969). Habitat loss and market hunting extirpated tule elk from most of their native range, causing conservationists to look for new areas in which to establish additional populations (McCullough, 1969). Although the elk population in Owens Valley has remained relatively stable, antler and bone abnormalities have been observed over many years. Clearly, this population is susceptible to problems not experienced by tule elk inhabiting native ranges, as antler breakage indicates that habitat conditions in Owens Valley may not be fulfilling nutritional requirements. Currently, the elk population in Owens Valley is one of the largest in California and has played a pivotal role in restoring this subspecies to areas from which it had been extirpated (McCullough et al., 1996). Wildlife managers will have to determine whether antler breakage poses a problem of ecological or public interest, and whether the population should be treated for nutritional deficiencies.

ACKNOWLEDGMENTS

We thank J. Koprowski for his helpful comments on this manuscript; B. Gonzales, A. Ellsworth, and J. Fischer for project support; P. McGrath and D. Racine for assistance with data collection in Owens Valley; and C. Fien for collecting elk samples at Grizzly Island Wildlife Area. T. Noon assisted with project design and facilitated mineral analysis through the University of Arizona Veterinary Diagnostic Laboratory. S. Archer supplied plant-grinding equipment. This study was funded by the Rocky Mountain Elk Foundation and the CDFG Elk Management Program. This is a contribution from the CDFG Elk Management Program and is Professional Paper 042 from the Eastern Sierra Center for Applied Population Ecology.

LITERATURE CITED

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. 1990. Official methods of analysis of the Association of Official Analytical Chemists. 15th Edition. Association of Official Analytical Chemists, Arlington, Virginia, 1298 pp.

———. 2000. Official methods of analysis of the Association of Official Analytical Chemists. 17th Edition. Association of Official Analytical Chemists, Shelton, Connecticut, 2200 pp.

AUDIGÉ, L., P. R. WILSON, R. S. MORRIS, AND G. W. DAVIDSON. 1995. Osteochondrosis, skeletal abnormalities and enzootic ataxia associated with copper deficiency in a farmed red deer (*Cervus elaphus*) herd. New Zealand Veterinary Journal 43: 70–76.

BARLOW, R. M. 1978. Enzootic ataxia of deer. *In* Symposium on the comparative pathology of zoo animals, R. S. Montali and G. Migaki (eds.). Smithsonian Institute Press, Washington D.C., pp. 65–71.

BLEICH, V. C. 1990. On calcium deficiency and brittle antlers. Journal of Wildlife Diseases 26: 588.

———, C. S. Y. CHUN, R. W. ANTHES, T. E. EVANS, AND J. K. FISCHER. 2001. Visibility bias and development of a sightability model for tule elk. Alces 37: 315–327.

BOBEK, B., K. PERZANOWSKI, AND J. WEINER. 1990. Energy expenditure for reproduction in male red deer. Journal of Mammalogy 71: 230–232.

CALIFORNIA DEPARTMENT OF FISH AND GAME. 1993. Trace element digestion of tissue. Fish and Wildlife Water Pollution Laboratory, Rancho Cordova, California, 8 pp.

COWEN, R. L., AND T. A. LONG. 1962. Studies on antler growth and nutrition of white-tailed deer. National White-tailed Deer Disease Symposium 1: 54–60.

CUNNINGHAM, I. J. 1950. Copper and molybdenum in relation to diseases of cattle and sheep in New Zealand. *In* A symposium on animal, plant and soil relationships, W. D. McElroy and B. Glass (eds.). The Johns Hopkins Press, Baltimore, Maryland, pp. 246–273.

DAVIS, G. K. 1950. The influence of copper on the metabolism of phosphorus and molybdenum. *In* A symposium on animal, plant and soil relationships, W. D. McElroy and B. Glass (eds.). The Johns Hopkins Press, Baltimore, Maryland, pp. 216–229.

DELHAIZE, E., J. F. LONERAGAN, AND J. WEBB. 1987. Copper in plants, its relation to soils and availability to animals. *In* Copper in animals and man, J. M. Howell and J. M. Gawthorne (eds.). CRC Press, Boca Raton, Florida, pp. 1–19.

FRANZMANN, A. W., J. L. OLDEMEYER, AND A. FLYNN. 1975. Minerals and moose. North American Moose Conference and Workshop 11: 2–26.

FRENCH, C. E. 1956. Nutrient requirements for growth and antler development in white-tailed deer. Journal of Wildlife Management 20: 221–232.

GOGAN, P. J. P., AND R. H. BARRETT. 1987. Comparative dynamics of introduced tule elk populations. Journal of Wildlife Management 51: 20–27.

———, D. A. JESSUP, AND R. H. BARRETT. 1988. Antler anomalies in tule elk. Journal of Wildlife Diseases 24: 656–662.

———, ———, AND M. AKESON. 1989. Copper deficiency in tule elk at Point Reyes, California. Journal of Range Management 42: 233–238.

GOSS, R. J. 1983. Deer antlers: Regeneration, function, and evolution. Academic Press, New York, New York, 316 pp.

GRACE, N. D., AND P. R. WILSON. 2002. Trace element metabolism, dietary requirements, diagnosis and prevention of deficiencies in deer. New Zealand Veterinary Journal 50: 252–259.

HAUFLER, J. B., AND F. A. SERVELLO. 1996. Techniques for wildlife nutritional analyses. *In* Research and management techniques for wildlife and habitats, T. A. Bookhout (ed.). Allen Press, Lawrence, Kansas, pp. 307–323.

HENSHAW, J. 1971. Antlers—The unbrittle bones of contention. Nature 231: 469.

HYVARINEN, H., T. HELLE, M. NIEMINEN, P. VAYRYNEN, AND R. VAYRYNEN. 1977. The influence of nutrition and seasonal conditions on mineral status in the reindeer. Canadian Journal of Zoology 55: 638–655.

JOHNSON, H. E., V. C. BLEICH, AND P. R. KRAUSMAN. 2005. Antler breakage in tule elk, Owens Valley, California. Journal of Wildlife Management 69: 1747–1752.

KUCHLER, A. W. 1977. A map of the natural vegetation of California. *In* Terrestrial vegetation of California, M. G. Barbour and J. Major (eds.). John Wiley & Sons, New York, New York, pp. 909–938.

LOGSDON, H. S. 1973. Food habits of the tule elk in Owens Valley, California. Final Report, Contract S-1426. California Department of Fish and Game, Sacramento, California, 58 pp.

MARTIN, R. B. 1991. Determinants of the mechanical properties of bones. Journal of Biomechanics 24 (Suppl. 1): 79–88.

MCCORQUODALE, S. M., L. E. EBERHARDT, AND G. A. SARGEANT. 1989. Antler characteristics in a colonizing elk population. Journal of Wildlife Management 53: 618–621.

MCCULLOUGH, D. R. 1969. The tule elk: Its history, behavior, and ecology University of California Publications in Zoology 88. Berkeley, California, 209 pp.

———, J. K. FISCHER, AND J. D. BALLOU. 1996. From bottleneck to metapopulation: Recovery of the tule elk in California. *In* Metapopulations and

wildlife conservation, D. R. McCullough (ed.). Island Press, Washington, D.C., pp. 375–403.

McDowell, L. R. 1985. Nutrition of grazing ruminants in warm climates. Academic Press, New York, New York, 443 pp.

———. 1992. Minerals in animal and human nutrition. Academic Press, San Diego, California, 524 pp.

National Oceanic and Atmospheric Administration. 2003. Climatological data annual summary, monthly surface data, California. National Climate Data Center, Ashville, North Carolina, http://cdo.ncdc.noaa.gov/ulcd/ULCD

Paynter, D. I. 1987. The diagnosis of copper insufficiency. *In* Copper in animals and man, Vol. 1, J. M. Howell and J. M. Gawthorne (eds.). CRC Press, Boca Raton, Florida, pp. 101–119.

Puls, R. 1994. Mineral levels in animal heath: Diagnostic data. 2nd Edition. Sherpa International, Clearbrook, British Columbia, Canada, 356 pp.

Robbins, C. T. 1993. Wildlife feeding and nutrition. 2nd Edition. Academic Press, New York, New York, 352 pp.

———, S. M. Parish, and B. L. Robbins. 1985. Selenium and glutathione peroxidase activity in mountain goats. Canadian Journal of Zoology 63: 1544–1547.

Rucker, R. B., H. E. Parker, and J. C. Rogler. 1969. The effects of copper on collagen cross-linking. Biochemical and Biophysical Research Communications 34: 28–33.

———, R. S. Riggins, R. Laughlin, M. M. Chan, M. Chen, and K. Tom. 1975. Effects of nutritional copper deficiency on the biomechanical properties of bone and arterial elastin metabolism in the chick. Journal of Nutrition 105: 1062–1070.

Samson, J., J. T. Jorgensen, and W. D. Wishart. 1989. Glutathione peroxidase activity and selenium levels in Rocky Mountain bighorn sheep and mountain goats. Canadian Journal of Zoology 67: 2493–2496.

Severinghaus, C. W., H. F. Maguire, R. A. Cookingham, and J. E. Tanck. 1950. Variations by age class in the antler beam diameters of white-tailed deer related to range conditions. North American Wildlife and Natural Resources Conference 15: 551–570.

Shupe, J. L., J. E. Butcher, J. W. Call, A. E. Olson, and J. T. Blake. 1988. Clinical signs and bone changes associated with phosphorus deficiency in beef cattle. American Journal of Veterinary Research 49: 1629–1636.

Stowe, H. D., W. E. Braselton, J. B. Kaneene, and M. Slanker. 1985. Multielement assays of bovine tissue specimens by inductively coupled argon plasma emission spectroscopy. American Journal of Veterinary Research 46: 561–565.

Suttle, N. F. 1975. The role of organic sulfur in the copper-molybdenum-S interrelationship in ruminant nutrition. British Journal of Nutrition 34: 411–420.

———, and K. W. Angus. 1978. Effects of experimental copper deficiency on the skeleton of the calf. Journal of Comparative Pathology 88: 137–148.

Underwood, E. J. 1977. Trace elements in human and animal nutrition. Academic Press, New York, New York, 545 pp.

———, and N. F. Suttle. 1999. The mineral nutrition of livestock. 3rd Edition. CABI Publishing, New York, New York, 614 pp.

Received for publication 27 May 2004.