Ovine White-Liver Disease (OWLD). Botanical and Chemical Composition of Pasture Grass

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Ulvund, M. J. and M. Pestalozzi: Ovine white-liver disease (OWLD). Botanical and chemical composition of pasture grass. Acta vet. scand. 1990, 31, 257–265. – The most important grass species on the ovine white-liver disease (OWLD) pastures (S) were *Poa* spp., *Agropyron repens* and *Lolium perenne,* while the control pastures (H), where lambs grew well, consisted of *Poa* spp., *Festuca rubra* and *Agrostis tenuis.* The soil was more acidic on the H pastures as compared with the S pastures. OWLD grass (S grass) contained marginal to deficient amounts of cobalt during the first 2 months of grazing. During 2 years out of 3, the average Co content was slightly lower in the S grass as compared with the content in the H grass. The lowest average grass Co was, however, found during one year in the H grass, in spite of the fact that the H lambs also this year grew well, and were 13 kg heavier than the S lambs after 3 1/2 months on pasture. Results thus indicate that the H lambs some years were subclinically Co deficient, without developing clinical symptoms or OWLD, and that factors other than marginal/deficient grass Co are of importance as to whether OWLD will develop or not. S grass differed from H grass by having significantly lower copper, molybdenum, manganese and zinc content, lower protein N/amid N ratios and higher aluminium and iron contents. According to the results, marginal to deficient grass Co is essential for development of OWLD, but cofactors play a part.

sheep; cobalt/vitamin B_{12} deficiency; trace elements.

Introduction

Clinical symptoms of ovine white-liver disease (OWLD) and the effect of prophylactic measures were described in a previous publication (*Ulvund & Pestalozzi* 1990). OWLD has been found in lambs grazing various grass species, either containing adequate amounts of cobalt (*Sutherland* 1978, *Clark et al.* 1978, *Richards & Harrison* 1981), or being marginal to deficient in Co (*Sutherland* 1978, *Sutherland et al.* 1979, *Mitchell et al.* 1982, *Mason & McKay* 1983 and *McLoughlin et al.* 1984). *Martinovich* (1974) suggested that OWLD might be due to mycotoxic effect in Co/B12 deficient lambs,

while *Sutherland et al.* (1979) proposed that high amounts of dietary precursors of propionic acid might be significant.

Plants containing mycotoxins or alkaloids may cause illthrift and hepatotoxicity in sheep (*Gardiner* 1965, *Seaman* 1987). Illthrift has occurred in lambs grazing reed canarygrass (*Phalaris arundinacea*) containing the alkaloid gramine (*Marten et al.* 1976). In our area, phalaris stagger in lambs grazing reed canarygrass has been described (*Ulvund* 1985).

It was therefore of interest to clarify the botanical and chemical composition of OWLD grass as well as grass from pastures where

lambs grow well, with special reference to trace elements and gramine.

Materials and methods

Experimental design has been described (*Ulvund & Pestalozzi* 1990). All pastures were located on morainic soil with sand as dominating fraction. The OWLD pastures (S) were on deep drought resistant top soil, on ploughed land, seeded 14–30 years ago. The control pastures (H), where lambs have been thriving well for years, were situated 15 km apart, and were on surface cultivated hilly terrain with varying humus content in the soil. The pastures were situated in southwestern Norway, 50–100 m above sea level, and 2–6 km from the sea.

Details of fertilization are given in Table 1. Each year, about 40 times as much calcium, 6 times as much sulphur, and twice as much nitrogen, phosphorous and potassium were used per ha on the S pastures as compared with the H pastures. Parts of the pastures were fertilized with Co (Table 2).

Table 1. Fertilization of pastures. Total amount of elements used each year on the OWLD pastures (S), and the control (H) pastures (kg/ha).

Element	S pasture	H pasture
N	205	130–180
P	30	15–20
K	100	35–45
Mg	13	6–8
S	60	8–10
Ca	370	8–10
Cu	0.7	0
B	0	0.03–0.04

On the S pastures 650 kg PK fertilizer (5–16) was used in early spring, and 700+500+400 kg calcium nitrate (15.5 % N) during the grazing season (May-October). The H pastures were fertilized with commercial NPK fertilizer (25-3-6) 3–4 times during the grazing season, and about 180 kg per ha was used each time.

Rainfall figures and temperatures were obtained from the nearest meteorological station Sola, 12 km from the S pastures, and 14 km from the H pastures. The range in mean monthly temperatures during the grazing period (May-September) for the years 1981–1986 was 8–15°C. The mean yearly temperature during these years was 7.1°C, which was close to the 30-year normal (1931–1960) of 7.4°C. Total annual precipitation varied from 979 to 1417 mm, overall mean was 1255 mm. The years 1981–1983 and 1986 had an annual rainfall above 1350 mm, while 1984–1985 had below 990 mm. The 30-year normal annual precipitation for the area is 1015 mm.

The pH of the soil was examined in 1978 and 1988. In 1978, pH was on average 5.2 (5.1–5.3) on the H pastures, and 5.5 on the S pastures, while ten years later, it was 4.7 (4.6–4.7) on the H pastures, and 6.1 on the S pastures.

The botanical composition was examined in 1978 by fencing off with cage net 6×1 m^2 on each pasture. The swards were cut in July, sorted in species and dried.

Grass samples for chemical analysis were taken 3 times during the grazing season (Table 2). Multiple small portions of grass from all quadrants of each pasture were cut and mixed, and approximately 1 kg from each plot was dried for 48 hours at 60°C. Efforts were made to avoid soil contamination. The samples collected in 1982 were frozen (– 20°C). For gramine estimations samples of 0.1 kg fresh grass were frozen.

Co and other metal concentrations were determined by simultaneous multi-element analysis in a d.c. plasma-atomic emission spectrometer by SVA-Uppsala (*Frank & Petersson* 1983). Ås-NLH used the following methods: Flameless atomic absorption spectrophotometry (AAS, grafite oven) for Cu determination, flame AAs for Mn determi-

Table 2. Survey of grass samples examined. Year of sampling, pasture of origin, parameters examined, and performing laboratory are given. Three different grass samples were taken from each pasture every year, during week 21 (May), 26 (June) and 32 (Aug.).

Year	Pasture	Parameters	Laboratory
1982	S, SCo, H	Mn, Cu, Mo, tot. S:	Ås-NLH
1983	S, SCo, H	Mn, Cu, Mo, tot. S, amino-acid-S, tot. N, prot. N:	Ås-NLH
1984	S, SCo, H	Mn, Cu, Mo, tot. S, Fe, tot. N, prot. N:	Ås-NLH
		Co:	SVA
1985	S, SCo, SCo+ ,H	Tot. N, prot. N, tot. S:	Ås-NLH
		Co, Mn, Cu, Fe, Zn:	SVA
		Mo, Se:	NVI
		P:	CVM
		Gramine:	SF-Fureneset
1986	S, SCo, SCo+ ,H	Co, Mn, Cu, Mo, Fe, Zn, Mg, Ca, Pb, Al, P, Tot. S:	SVA
	HCo	Gramine:	SF-Fureneset

The OWLD pastures are designated S. SCo are S pastures Co fertilized in 1980, 1981 and 1982, while SCo+ pastures were Co fertilized 1980, 1981, 1982, 1985 and 1986 (CoSO$_4$, 1 kg/ha). Control pastures are designated H, and HCo are H pastures Co fertilized in 1986 (CoSO$_4$, 2 kg/ha).

Ås-NLH : Chemical Research Laboratory, Agricultural University of Norway.
SVA   : National Veterinary Institute, Uppsala, Sweden.
NVI   : National Veterinary Institute, Oslo, Norway.
CVM   : Department of Biochemistry, Norwegian College of Veterinary Medicine, Oslo, Norway.
SF   : Fureneset Research Station, Norwegian State Agricultural Research Stations, Fure, Norway.

nation, Mo was examined by spectrophotometry, total sulphur by spectrophotometric determination of turbidity (BaSO$_4$-suspension), Kjeldahl-N according to Kjeldahl's method modified for Tecator Kjeltec with CuO as catalyst, and pure protein-N according to Barnstein-Stutzer method modified for Tecator Kjeltec (*Halvorsen* 1989). Co analyses were also performed in 1981–1983, with flameless AAS, but because of technical reasons the results were unreliable and had to be excluded. NVI-

Oslo used flameless AAS for the determination of molybdenum, and a fluorimetric method for the determination of selenium (*Norheim* 1989). Gramine was examined according to *Simons & Marten* (1971), as described by *Østrem* (1987).

Blind parallels were included and the divergence in results was within acceptable limits. Estimates of the Cu availability (A$_{Cu}$, %) and absorbability (Abs$_{Cu}$) were made according to *Suttle* (1983). Statistical testing of differences was made by one way analysis of var-

iance (*Snedecor & Cochran* 1967). Least significant difference (LSD) was calculated only when F was significant.

Table 3. Cobalt contents (mg/kg DM, activation analysis) in pasture grass sampled in week no. 21, 26, and 32 for the years 1984–1986. For explanation of pasture symbols, see footnote of Table 2.

Year	Week no.	S	SCo	SCo+	H	HCo
1984	21	0.15	0.16	–	0.24	–
	26	0.13	0.18	–	0.17	–
	32	0.16	0.22	–	0.27	–
1985	21	0.10	0.47	0.45	0.16	–
	26	0.08	0.16	0.28	0.09	–
	32	0.11	0.22	0.39	0.21	–
1986	21	0.09	0.12	1.70	0.09	1.34
	26	0.09	0.14	0.37	0.10	1.05
	32	0.17	0.15	0.32	0.06	0.64

Results

The most important grass species on the S pastures were *Poa* sp. (39 %), *Agropyron repens* (27 %) and *Lolium perenne* (16 %), and on the H pastures *Poa* sp. (68 %), *Festuca rubra* (11 %) and *Agrostis tenuis* (10 %). During 2 years out of 3, the average Co content was slightly lower in the S grass as compared with the H grass (Table 3). The lowest average grass Co was, however, found during 1 year (1986) in the H grass. Fertilizing the pastures with Co increased the content substantially the first years after treatment, but the effect decreased after 4 years.

The contents of Mn, Cu, Mo and Zn was significantly higher in H grass than in S grass, while the content of Al, Fe and Ca was highest in S grass (Table 4). The Cu/Mo ratios of the S grass were not significantly

Table 4. Total number of grass samples examined (n) from the S, SCo and H pastures and overall mean amount (in DM) of Mn, Cu, Mo, Fe, Zn, Al (mg/kg), Ca (g/kg), approximate Cu availability (ACu, %) and protein N/amid N. Significance and least significant difference between these three pastures (LSD, 5 per cent), and significance of difference in sampling time are given. Mean values for samplings (S, SCo, H) performed during the same week each year are also given. For explanation of pasture types and years of sampling, see Table 2.

	Mn	Cu	ACu	Mo	Fe	Zn	Ca	Al	ProtN/AmidN
n	54	54	39	54	36	27	15	15	30
S	53	4.6	3.1	1.0	186	33	6.7	143	3.1
SCo	47	4.8	2.8	1.2	189	32	6.3	91	3.5
(SCo+	57	4.5	2.9	1.1	144	32	5.9	61	3.3)
H	133	6.8	2.3	1.6	106	40	3.3	29	4.5
(HCo	89	5.8	–	1.2	88	32	4.0	30	–)
Sign.	***	***	**	***	*	*		*	**
LSD	23	0.7	0.4	0.2	68	6	ns	78	0.6
May	62	5.6	2.2	1.3	132	34	5.0	47	3.8
June	68	4.5	3.8	1.1	114	32	4.1	59	3.5
Aug.	94	5.7	2.3	1.4	206	36	6.7	106	3.8
Sign.	**	***	***	**	**	ns	ns	ns	ns

For SCo+ and HCo (in brackets), adjusted mean values only represent samplings from one or two years.
Sign. = Significance levels, ns > 5, * < 5, ** < 1, *** < 0.1 per cent.

Table 5. Kjeldahl N, total sulphur (g/100 g DM), amino acid S, P (g/kg DM), Mg (mg/kg DM), Se, AbsCu (µg/kg DM) and gramine (µg/g DM) in pasture grass. n = total number of grass samples examined. Mean values ± SD for all pastures and years are given.

	Kjel-dahl N	Tot. S	Amino-acid S	P	Mg	Se	AbsCu	Gramine
n	30	54	9	27	15	12	45	31
mean	35	29	1.7	3.6	1.6	16.6	140	70
± SD	0.8	8.0	0.2	0.7	0.2	10.0	50	43

different from the ratios of the H grass, the overall values were 4.9 (S) as compared to 4.7 (H). The availability of Cu (A_{Cu} %) was significantly higher in S grass, while the calculated absorbable amount (Abs_{Cu}) varied much in both S and H grass (Tables 4 and 5).

Fertilizing with Co decreased grass Al, and partly also Ca. The protein N to amid N ratios were lower in S grass than in H grass, and Co fertilization seemed to increase the ratio (Table 4). The overall mean amount of Pb was 0.33 mg/kg in S grass, 0.27 in moderately Co fertilized S grass (SCo) and 0.18 in heavily Co fertilized S grass (SCo+). Corresponding amounts in H and HCo grass were 0.68 and 0.61 mg/kg.

There were no significant differences between S and H grass regarding the content of Kjeldahl N, total S, amino acid S, P, Mg, Se, AbsCu and gramine. Overall mean values of these elements are given in Table 5. The overall pure protein N content was 3.0 (g/100 g DM) in H grass, and 2.7 and 2.5 in S and SCo grass respectively. The lowest amounts of most elements were found during week 26 (June, Table 4).

Discussion

Sandy soil, rainy climate, leafy pastures, dead litter and freely drained soil were characteristics of our S pastures. These features have also been associated with OWLD in other countries (*Clark et al.* 1978, *Sutherland et al.* 1979, *Mason & McKay* 1983), as has pure Co deficiency (Andrews et al. 1958, *Robertson* 1971). The grass of the H pastures was less lush, and the soil poorer drained. The OWLD condition was mostly pronounced in 1981–1983 and 1986 (*Ulvund & Pestalozzi* 1990), when the annual precipitation was highest.

OWLD has been found in lambs grazing ryegrass, *Lolium perenne*, (*Mason & McKay* 1983), ryegrass, white clover (*Trifolium repens*) and kikuyu grass (*Pennisetum clandestinum, Sutherland et al.* 1979), or ryegrass, oat stubble, clover (*Trifolium repens*) and kikuyu grass (*Richards & Harrison* 1981). Common for these and our OWLD pastures was the perennial ryegrass. Ryegrass may contain alkaloids (*Grimmett & Melville* 1943), mycotoxins (*Siegel et al.* 1985) or bacteria and nematodes producing hepato-/neurotoxins (*Berry et al.* 1982). Further examination of our ryegrass could therefore be a next step.

It is generally accepted that sheep require 0.11 mg Co/kg DM, and mixed herbage on soil classified as adequate in Co normally contains > 0.10 mg/kg (*Anon.* 1980, *Anon.* 1982, *Smith* 1987). If the herbage consistently contains less than 0.08 mg Co, Co/B12 deficiency will develop (*Smith* 1987). The Co content in our OWLD grass may thus be classified as normal to margi-

nal/deficient, with marginal/deficient values in June each year. However, marginal/deficient values were also found in H grass. In 1986, when the Co content in H grass was lower than in S grass, the growth of the H lambs was on average 4 kg less than that of the 2 preceding years, indicating a subclinical Co deficiency (*Ulvund & Pestalozzi* 1990). But still they were 13 kg heavier than the S lambs by September 1, and none developed clinical OWLD.

During later years (1987, 1988), the average Co content was also lower in H grass as compared with S grass, while the lambs grew very well (*Ulvund & Pestalozzi,* unpublished). Thus it can be concluded that OWLD is not solely due to scarce grass Co. On the S pasture there was a certain correlation between Co content in the grass during the first 2 months of grazing and seriousness of OWLD. Lambs grazing H grass with similar Co values were, however, healthy and normally growing. The difference in growth rate between H and S lambs, and the development of OWLD was therefore not due to varying degree of simple Co deficiency within the grass.

Sutherland (1978) reported 8 outbreaks of OWLD in New Zealand, where none of the pastures contained more Co than 0.10 mg/kg, while *Clark et al.* (1978) found OWLD on pastures containing more than 0.16 mg Co/kg. In Australia, OWLD has been reported on pastures with normal Co contents (*Richards & Harrison* 1981), marginal amounts 0.09–0.13 mg/kg (*Mason & McKay* 1983), and on pastures being deficient 0.02 mg/kg (*Mitchell et al.* 1982). In Northern Ireland, OWLD occurred on a pasture containing 0.08 mg Co/kg (*McLoughlin et al.* 1984).

Liming and high soil content of Mn and Fe may decrease the availability of soil Co to the grass, and an increase in soil PH may also reduce Co uptake (*Anon.* 1982, *McLaren et al.* 1987). The extensive use of Ca as well as the higher soil pH on the S pastures may therefore be of some significance regarding the Co content of the S grass. Simple Co deficiency is known to occur on soil rich in Ca (*Robertson* 1971), and coast disease, a concurrent deficiency of Co and Cu, occurred on calcareous sandy soil containing windborne shell fragments in South Australia (*Marston et al.* 1938). Little is known about the details in this Co/Ca interaction. As, however, the liver content of Co was equally marginal to deficient in both our OWLD lambs (S lambs) and H lambs (*Ulvund* 1990a), any significant role of Ca in the present material is so far difficult to interpret.

The use of Ca on the S pastures may have reduced the amount of grass Mn as well. *Mitchell et al.* (1957) reported that liming of ryegrass reduced the mean Mn value from 140 to 120 mg/kg. Any inhibitory effect of Mn on Co availability can probably be ruled out in our case. As minimum requirements of growing lambs are estimated to be about 20–25 mg Mn/kg (*Anon.* 1980, *Anon.* 1982), our S lambs probably got sufficient Mn. High intakes of Ca in the diet may, however, have interfered with the intestinal absorption (*Hurley & Keen* 1987), explaining the lower liver Mn seen in S lambs during some years (*Ulvund* 1990a).

High amounts of Fe may inhibit Co absorption from the intestine (*Smith* 1987). The low liver Co found in both H and S lambs indicates that the higher Fe content in S grass had no significance (*Ulvund* 1990a). *Halpin et al.* (1982) reported that sheep grazing plots receiving high applications of super phosphate had decreased plasma B_{12}. The lack of difference between S and H grass in P content indicates that this effect cannot explain the occurrence of OWLD.

Variations in Cu absorbability is great, and dependant on Mo, S, Fe, Zn, Ca and breed (*Davis & Mertz* 1987). In coastal districts in Norway, mean value of Cu in cultivated herbage was found to be 6.9 mg/kg DM (*Frøslie & Norheim* 1983). Grass Cu decreases by liming, especially if the original content is scarce (*Anon.* 1982). This may have contributed to the lower Cu content in our S grass. Liver Cu was often deficient in the OWLD lambs, and most often lower than in the H lambs, although individual H lambs also had deficient amounts (*Ulvund* 1990a). A role of Cu in the OWLD pathogenesis can therefore not be excluded, but is discussed in detail elsewhere (*Ulvund* 1990a, b, c).

Grass Mo in our pastures were within similar ranges as reported by *Frøslie & Norheim* (1983), who found a mean value of 0.8 mg/kg DM in samples from coastal areas. Grass Mo may increase by liming and pasture improvement (*Anon.* 1982). *Mills & Davis* (1987) report 0.2–0.8 mg/kg as representative amounts in grasses, with increased amounts concomitant with increases in soil pH. The fact that S grass contained less Mo than H grass, in spite of higher soil pH, probably reflects much lower soil Mo on the S pastures.

The Cu/Mo ratios varied between 3 and 9.5 in our material. A balanced diet often contains a Cu/Mo ratio of 5–10, with a lower critical ratio of 2, and an upper critical ratio of 20 (*Frøslie & Norheim* 1983).

The amount of sulphur found in our pastures was slightly higher than the amount found by *Frøslie & Norheim* (1983) in coastal grass. The increase may be due to pasture improvement (*Anon.* 1982). The A_{Cu} and Abs_{Cu} of our pastures were of the same magnitude as those recorded in summer pastures in Scotland (*Suttle* 1983).

Minimum requirements of Zn are about 15 mg Zn/kg DM (*Anon.* 1982). Our lambs should therefore be provided with enough Zn. Norwegian coastal pastures on average contained 39 mg Zn/kg DM (*Frøslie & Norheim* 1983), which was similar to contents in Scottish herbage (*Anon.* 1982).

Grass Al has become more soluble in surface waters and is easier absorbed by the plants as a result of acid rain (*Alfrey* 1986). Al may interfere with the absorption of several elements, cause neurotoxicity and a microcytic hypochromic anaemia (*Alfrey* 1986). So far it is difficult to interpret a role of Al in the pathogenesis of OWLD.

The amount of Pb found in our samples was within normal limits (*Quarterman* 1986). It is unknown whether the reduced protein N to amid N ratios in OWLD grass may have any significance in the development of the disease. The lack of difference in gramine contents between S and H grass probably rule out these alkaloids as cofactors of the OWLD pathogenesis. The lack of difference regarding the other elements given in Table 5 probably also indicates that these are not significant.

The lower contents of trace elements found in the grass in June each year probably reflect the high grass yield resulting in an effect of dilution.

OWLD was associated with pasture grass containing marginal/deficient Co during the first months of grazing. Similar Co values were, however, also found in other pastures, where clinically healthy lambs gained 13 kg more than the OWLD affected ones during the grazing period. Outbreaks of OWLD are therefore dependant on cofactors. The differences found between S and H grass regarding other metal concentrations, especially Cu, may be of significance.

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Sammendrag

Kvitleversjuke (kobolt/vitamin B₁₂ mangel) hos lam. Botanisk og kjemisk sammensetning av beitegraset.

De viktigste grasartene på sjukdomsbeitene (S) var rapparter (*Poa* spp.), kveke (*Agropyron repens*) og vanlig raigras (*Lolium perenne*), mens kontrollbeitene (H), der lamma hvert år har svært god tilvekst, besto av rapp, rødsvingel (*Festuca rubra*) og engkvein (*Agrostis tenuis*). Jorda var surere på H-beitene enn på S-beitene. S-graset inneholdt marginale til deficitte koboltmengder i første del av beitesesongen. I 2 av 3 år hadde S-graset lavere koboltinnhold enn H-graset, men 1 år var koboltinnholdet like lavt i H-graset. Dette året vokste H lamma likevel 13 kg mer iløpet av beitesesongen enn S lamma. Resultatene indikerer at det må ha vært subklinisk koboltmangel hos H lamma visse år, og at opptreden av OWLD ikke bare er avhengig av lavt Co innhold i beitet. S-graset hadde signifikant lavere kopper-, molybden-, mangan- og sinkinnhold, lavere protein N/amid N, og høyere aluminium- og jerninnhold. Resultatene viser at marginale koboltmengder eller mangelnivå i graset er av betydning for utvikling av kvitleversjuke, men at interferens av andre faktorer spiller en rolle.