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# THE EFFECT OF DIFFERENT LEVELS OF SELENIUM IN MINERAL MIXTURES AND SALT LICKS ON SELENIUM STATUS IN SHEEP

By

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ØVERNES, GUNNAR, KNUT MOKSNES, ARNE FRØSLIE, JO GUNNAR NØRSTEBØ and JOHANNES FLAAT: *The effect of different levels of selenium in mineral mixtures and salt licks on selenium status in sheep.* Acta vet. scand. 1985, *26*, 405—416. — This paper describes 3 experiments comparing the effect of 10, 25 and 40 mg Se/kg, as sodium selenite, in mineral mixtures and salt licks fed to sheep. The supplement was given during the indoor season from October to May to 7 different flocks, each consisting of 50 to 100 sheep, in areas with selenium deficiency problems. The average selenium level in the basic diets did not exceed 0.05 mg/kg. Selenium status was monitored in the blood of ewes and lambs, and in milk. Blood selenium in lambs correlated well with blood selenium in their dams (r = 0.85). Selenium levels in milk on day 1 (colostrum) correlated well with selenium levels in dams (r = 0.92) and in offspring (r = 0.87). Statistically significant differences were found between the different flocks. In areas with extreme selenium deficiency, 10 mg Se/kg in mineral mixtures and salt licks proved insufficient. A content of 25 mg Se/kg, providing a daily intake of about 0.4 mg selenium, resulted in selenium levels in ewes' blood, ewes' milk and in the offspring that should prevent selenium deficiency disease without causing any toxic effects.

nutritional muscular dystrophy; prevention; feed additives.

Though different methods have been employed to provide supplementary selenium to animals fed mainly on roughages the problem of preventing nutritional muscular dystrophy (NMD) under field conditions has not yet been satisfactory solved.

In Norway, injections of sodium selenite was the only method available up to 1980, at which time commercial mixtures of concentrates and mineral feeds were fortified with selenium at a rate corresponding to 0.15 mg Se/kg in the compound concentrates (*Anon.* 1979). A slight decline in the number of cases of clinical NMD in sheep has been observed since 1980, but about 4000 cases are still reported annually (*Anon.* 1984). The addition of selenium to feeds thus seems to have exerted only a minor effect on the incidence of NMD in sheep. This lack of effect may be due to the addition of an insufficient amount of selenium, or to low consumption of concentrates and supplementary feeds.

The present experiments were carried out to study the effect of different levels of selenium in mineral mixtures and salt licks on selenium status in sheep and in their offspring.

## MATERIALS AN METHODS

The experiments were carried out during the indoor season from October to May.

The experimental design is listed in Table 1. In Experiment 1 a and b the sheep were given selenium at different levels incorporated into a standard mineral mixture (see Table 2) dosed daily at a rate of 15 g/sheep. In Experiment 2 salt licks composed of 96—98 % sodium chloride and 2—4 % magnesium chloride casted into 10 kg stones given ad libitum were used. The

Table 1. Experimental outline.

| Experiment | Indoor-season (year) | Calculated Se-content of the diet (mg/kg D.M.)[1] | Type and amount of supplement | Se-content of the supplement (mg/kg D.M.) | Estimated daily intake (µg Se/sheep) |
|---|---|---|---|---|---|
| 1a | 1982/83 | 0.05 | Mineral mixture 15 g/sheep/day | Flock 1: 10 | 200 |
| | | | | „ 2: 40 | 650 |
| | | | | „ 3: 40 | 650 |
| 1b | 1983/84 | | | Flock 1: 10 | 200 |
| | | | | „ 2: 25 | 425 |
| | | | | „ 3: 40 | 650 |
| 2 | 1983/84 | 0.10 | Saltlick ad lib. | Control[2] | 250 |
| | | | | Flock 1: 10 | 350 |
| | | | | „ 2: 25 | 725 |
| | | | | „ 3: 40 | 580 |
| 3 | 1983/84 | 0.05 | No supplement | Group 1: 10 | 50 |
| | | | Saltlick ad lib. | „ 2: 25 | 675 |
| | | | Mineral mixture 15 g/sheep/day | „ 3: 25 | 425 |

[1] Higher Se-content towards the end of the experiment.
[2] Mineral mixture 15 g/sheep/day containing 10 mg Se/kg.

Table 2. Composition of the mineral mixture.

| | | | |
|---|---|---|---|
| *Composition:* | | *Contents:* | |
| Limestone | 19.0 % | Calcium | 19 % |
| Monocalcium phosphate | 20.0 % | Phosphorus | 10 % |
| Dicalcium phosphate | 32.0 % | | |
| Sodium chloride | 23.8 % | Sodium | 10 % |
| Magnesium oxide | 4.0 % | Magnesium | 2 % |
| *Micro minerals added:* | | | |
| Manganese (II) oxide | | Manganese | 2,000 mg/kg |
| Zinc oxide | | Zinc | 2,700 „ |
| Calcium iodate | | Iodine | 130 „ |
| Cobalt (II) sulphate monohydrate | | Cobalt | 15 „ |
| Sodium selenite | | Selenium | 10, 25 or 40 mg/kg |

salt licks were added different amounts of sodium selenite. In Experiment 3 mineral mixture and salt licks containing the same amount of selenium were compared to an unsupplemented group.

*Experiment 1* was carried out in Numedal, a district known to have low selenium levels and with a history of NMD. Basic feed consisted of hay, silage and small amounts of compound concentrates. The feed were analysed for selenium and the total diet did not exceed 0.05 mg Se/kg. The flocks were sized between 50 and 100 sheep. Ten sheep from each flock were picked at random and eartagged for consecutive sampling of blood and milk. Blood samples were also drawn from one of their offspring. Blood samples from the ewes were drawn at the beginning of the experiments and throughout the indoor season. Samples of colostrum/milk were taken before suckling and on day 1, 3 and 7 after birth. Blood samples from lambs were drawn up to several times the first five weeks after birth. Experiment 1 continued for a period of 2 seasons including the same ewes.

*Experiment 2* was carried out in Alvdal, a district similar to Numedal, but with a more intensive feeding of compound concentrates especially during the periodes of tupping and partus. This brought the amount of selenium in total basic feed up to 0.10 mg/kg as an average during the indoor season. The control flock in this experiment did not receive salt licks, but a standard mineral mixture containing 10 mg Se/kg. This was a field trial and no unsupplemented groups were available out of fear of deficiency disorders. Sampling procedures were the same as in Experiment 1.

*Experiment 3* was carried out at a farm located near Oslo comprising 900 sheep. Though roughages in this area contain a slightly higher level of selenium than in Numedal and Alvdal, the farm had a history of NMD. The basic feed consisted of hay, silage and barley. Selenium content in basic feed did not exceed 0.05 mg/kg. One month before the lambing season all sheep received 0.5 kg of compound concentrates (0.40 mg Se/kg) per day. There were 30 sheep in each group and 10 were randomly tagged for sampling.

*Analyses*

Selenium levels in heparinized blood samples and milk were determined by a fluorimetric method (*Ihnat* 1974) after wet digestion (*Norheim & Nymoen* 1981), the same method being used to determine selenium in the roughages and concentrates used in the experimental flocks. Heparinized blood samples collected in experiment 1 in 1982/83 were also analysed for glutathione peroxidase activity according to the method of *Paglia & Valentine* (1967) using cumene hydro-peroxide as substrate. Statistical calculations were performed according to *Nissen* (1982).

## RESULTS

No cases of NMD occurred in the experimental flocks during the experimental period.

There was a very strong correlation between blood selenium and blood glutathione peroxidase activity in experiment 1 a ($r = 0.96$) as illustrated in Fig. 1. The analyses of selenium status were therefore limited to blood selenium in Experiment 1 b, 2 and 3, and only the blood selenium levels are reported in the present paper.

Results from Experiment 1 are listed in Table 3. At the beginning of the experiment, all three flocks had blood selenium levels well below 0.10 μg Se/ml. They quickly responded to selenium supplementation, and after eight weeks, average blood selenium levels in flock 1, flock 2 and flock 3 were 0.17, 0.18 and 0.20 μg Se/ml, respectively. Values in flock 1 seemed to level off at this point, while those in flocks 2 and 3 continued to rise until the end of the indoor season, reaching, in April just prior to lambing, an average of 0.33 μg Se/ml. In Oct. 1983, after 5 months on pasture with no selenium supplement, blood selenium levels had decreased to those of the previous year. The results

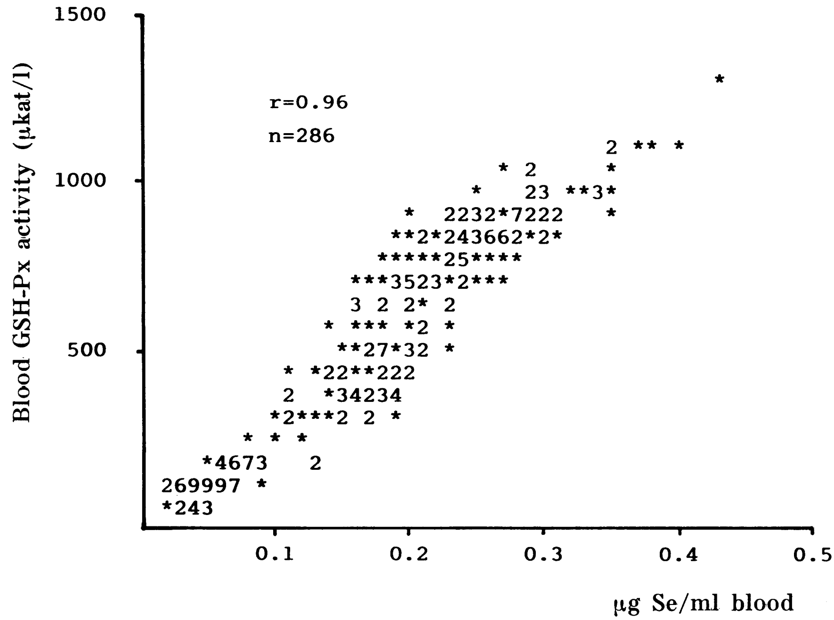


Figure 1. The relation between blood selenium and blood glutathione peroxidase activity in sheep. The figures indicate number of coinciding points.

Table 3. Levels of selenium (μg/ml) in blood of ewes and their lambs (means and standard deviations) after supplementation with selenium-enriched mineral mixture. Dosage: See Table 1, experiment 1.

| Flock | Nov.-82 | Jan.-83 | Mar.-83 | Apr.-83 | Lambs May-83 |
|---|---|---|---|---|---|
| 1 | 0.06±0.01 | 0.17±0.02 | 0.17±0.03 | 0.18±0.03 | 0.16±0.03 |
| 2 | 0.08±0.01 | 0.18±0.01 | 0.26±0.03 | 0.33±0.04 | 0.23±0.02 |
| 3 | 0.06±0.01 | 0.20±0.02 | 0.26±0.04 | 0.33±0.05 | 0.29±0.07 |
| Flock | Oct.-83 | | Mar.-84 | Apr.-84 | Lambs May-84 |
| 1 | 0.06±0.02 | | 0.14±0.03 | 0.15±0.03 | 0.12±0.03 |
| 2 | 0.08±0.02 | | 0.28±0.03 | 0.32±0.03 | 0.25±0.03 |
| 3 | 0.06±0.01 | | 0.33±0.05 | 0.38±0.06 | 0.29±0.05 |

in 1983/84 resembled those in 1982/83. Flock 1 levelled off at 0.15 μg Se/ml, while levels in flock 3 receiving a mineral mixture containing 40 mg Se/kg were significantly higher than in flock 2 receiving a mixture containing 25 mg Se/kg, blood selenium levels being 0.38 and 0.32 μg/ml, respectively. Selenium levels in lambs blood reflected those of the dams.

In Experiment 2 (Table 4), in which the salt licks were given ad libitum, larger standard deviations were observed due to larger differences in individual intake. Though flock 1 consisted of 2 different breeds, "Spel" and "Dala", there was no significant difference between the 2 breeds. At the start of the experiment, flocks 1 and 2 had already been receiving a mineral mixture containing selenium, initial base levels therefore being higher. Around lambing, after 5 months of feeding, selenium levels in flock 1 had declined slightly to around 0.18 µg Se/ml, while those in flock 2 were significantly higher at 0.35 µg Se/ml. Levels in flock 3 given a salt lick with 40 mg Se/kg, did not increase as much as expected. This particular flock of the "Pels-sau" breed ("Fur-breed") consumed only half of the anticipated amount of salt lick. The control group in this study received a regular supplement of 15 g standard mineral mixture containing 10 mg Se/kg and had blood selenium levels of 0.21 µg/ml. Levels in flock 1 did not exceed those in the control group, while those in flocks 2 and 3 did. Values in the lambs in flock 2 (0.23 µg Se/ml blood) were significantly higher ($P < 0.001$ and $P < 0.05$) than those in the lambs of flocks 1 (0.15 µg/ml) and 3 (0.19 µg/ml). There were no significant difference between flocks 1 and 3.

Table 4. Levels of selenium (µg/ml) in blood of ewes and their lambs (means and standard deviations) after supplementation with selenium-enriched salt lick. Dosage: See Table 1, experiment 2.

| Flock | Nov.-83 | Jan.-84 | Mar.-84 | Apr.-84 | Lambs May-84 |
|---|---|---|---|---|---|
| Control | | 0.21±0.04 | 0.21±0.08 | 0.21±0.08 | |
| 1 | 0.22±0.06 | 0.24±0.06 | 0.19±0.06 | 0.18±0.06 | 0.15±0.04 |
| 2 | 0.24±0.03 | 0.31±0.03 | 0.37±0.03 | 0.35±0.03 | 0.23±0.04 |
| 3 | 0.13±0.08 | 0.24±0.07 | 0.23±0.10 | 0.25±0.08 | 0.19±0.06 |

In Experiment 3 (Table 5) blood levels in the groups at the beginning of the experiment ranged from 0.11—0.14 µg Se/ml. Levels in the mineral mixture and salt lick groups rose quickly to about 0.25 µg/ml. Values in the mineral mixture group continued to increase, exceeding 0.35 µg Se/ml, while those in the salt lick group levelled off at around 0.25 µg Se/ml. Levels in the control group remained stable throughout the winter, averaging 0.13 µg/ml. The effect of feeding concentrates a month prior to lambing was recorded as a prompt rise in blood selenium levels, most noticeable in the control groups. The selenium levels in

Table 5. Levels of selenium (μg/ml) in blood of ewes and their lambs (means and standard deviations) after supplementation with selenium-enriched salt lick (group 2) and selenium-enriched mineral mixture (group 3). Dosage: See Table 1, experiment 3.

| Group | Dec.-83 | Feb.-84 | Mar.-84 | May-84 | Lambs May-84 |
|---|---|---|---|---|---|
| 1 | 0.14±0.02 | 0.13±0.02 | 0.13±0.03 | 0.20±0.04 | 0.14±0.02 |
| 2 | 0.11±0.01 | 0.25±0.05 | 0.22±0.04 | 0.26±0.05 | 0.19±0.04 |
| 3 | 0.11±0.02 | 0.31±0.05 | 0.33±0.04 | 0.36±0.04 | 0.23±0.03 |

lambs reflected those of the dams, though the difference between the groups were less significant. This may be partly explained by the heavy feeding around lambing in all groups.

All lambs in the last 3 experiments were sampled 2 or 3 times during their first 5 weeks of life. Blood selenium levels were stable throughout this period. Blood selenium in lambs correlated well with blood selenium in their dams (r = 0.85 Fig. 2).

Selenium values in milk are listed in Table 6. Values in milk on day 1 (colostrum) correlated well with blood selenium levels

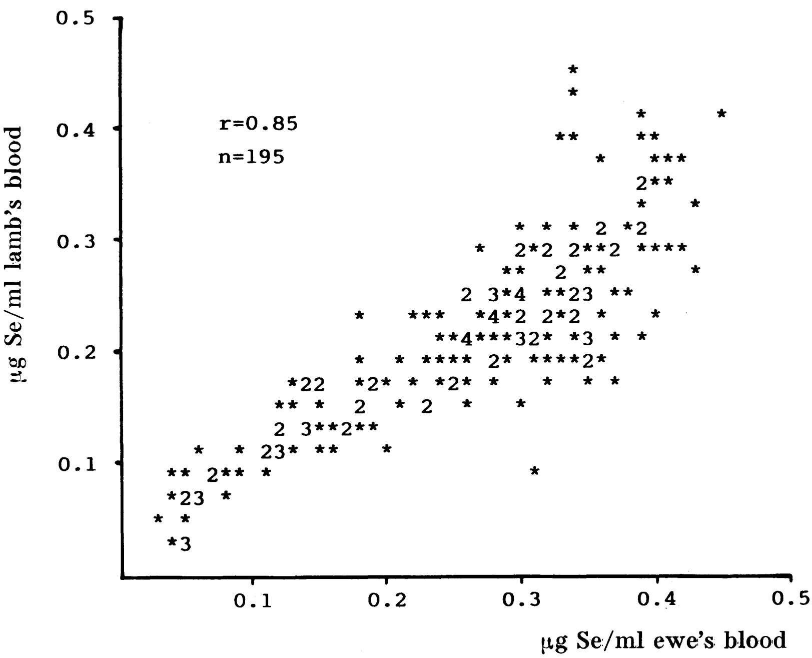


Figure 2. The relation between blood selenium in ewes and their offspring. The figures indicate number of coinciding points.

Table 6. Levels of selenium (μg/ml) in colostrum on day 1 after parturition compared to selenium levels in the ewes' blood prior to lambing after supplementation with selenium-enriched salt lick or mineral mixture. Means and standard deviations are given. Dosage: See Table 1.

| | Experiment 1a | | | Experiment 2 | |
|---|---|---|---|---|---|
| Flock | Ewes' blood | Colostrum | Flock | Ewes' blood | Colostrum |
| 1 | 0.18±0.03 | 0.03±0.01 | Contr. | 0.14±0.03 | 0.11±0.04 |
| 2 | 0.33±0.04 | 0.15±0.07 | 1 | 0.12±0.03 | 0.06±0.01 |
| 3 | 0.33±0.05 | 0.20±0.05 | 2 | 0.23±0.05 | 0.13±0.09 |
| | | | 3 | 0.19±0.06 | 0.07±0.02 |

| | Experiment 1 b | | | Experiment 3 | |
|---|---|---|---|---|---|
| Flock | Ewes' blood | Colostrum | Group | Ewes' blood | Colostrum |
| 1 | 0.15±0.03 | 0.04±0.04 | 1 | 0.20±0.04 | 0.08±0.06 |
| 2 | 0.32±0.03 | 0.12±0.04 | 2 | 0.26±0.05 | 0.07±0.05 |
| 3 | 0.38±0.06 | 0.31±0.15 | 3 | 0.36±0.04 | 0.10±0.07 |

in dams (r = 0.92) and in offspring (r = 0.87). This is based on samples taken before the lambs were allowed to suckle. In Experiment 1 a flocks 2 and 3 receiving the 40 mg/kg mixture, exhibited significantly higher levels than the 10 mg/kg flock. In Experiment 1 b, all three flocks were significantly different. In Experiment 2, the milk selenium levels reflected those of the blood levels. In Experiment 3, the group receiving mineral mixture showed the highest levels, but the heavy concentrate feeding around lambing evened out a possible difference between the groups.

On day 3 and 7, milk selenium declined drastically in all experiments, but the high-supplementary groups were still slightly higher (means from 0.03 to 0.05 μg/ml) than the low-supplementary groups (means of 0.02 to 0.03).

## DISCUSSION

According to *Blood et al.* (1983), normal or adequate serum selenium levels in sheep are from 0.08 to 0.5 μg/ml. The assumption should thus be that whole blood selenium levels have to be above 0.2 μg/ml to give sufficient protection against NMD. To obtain such levels in flocks in low selenium areas fed extensively on roughages, selenium has to be provided at a level above the minimum requirements of 0.1—0.2 mg Se/kg in the total diet. The "accumulated" deficit from the pasture season also requires that additional amounts of selenium should be supplied during the indoor feeding period to build up sufficient depots in ewes as they approach lambing. In areas of extreme selenium deficiency, the provision of the standard mineral mixture containing 10 mg Se/kg may therefore be inadequate. The use of mixes containing 25 mg Se/kg will result in selenium levels in ewes' blood, ewes' milk and in the offspring of a magnitude that should protect against selenium deficiency disease. The small differences in blood selenium values between mixes containing 25 and 40 mg/kg found in the present investigation indicate that the level of 25 mg/kg is sufficient. Where the use of salt licks is a more practical method of supplementation, a level of 25 mg Se/kg should be preferred. Salt licks can also provide selenium during the outdoor season.

In spite of the apparently higher intake of salt licks compared to mineral mixture, the potency of the selenium enriched salt licks is lower than that of the mineral mixture. The larger variation in blood levels seen with salt licks is probably due to a variation in intake. A level of 10 mg Se/kg in salt licks had no siginificant effect on the selenium status in sheep on ordinary feed. Though the effect of 25 mg Se/kg in salt licks was less than that produced by the same level of selenium in mineral mixture, it was still quite satisfactory in relation to critical blood selenium levels. It is difficult to pinpoint the reason for the lower potency of salt licks. Wastage while licking could be a cause, but it seems that the availability of selenium in salt licks may be inferior to that in mineral mixtures.

The insufficiency of levels of 10 mg Se/kg in mineral mixtures and salt licks is also supported by the analyses of milk. Milk levels in sheep receiving 10 mg Se/kg showed only slightly higher levels than sheep receiving no supplement at all, respectively 0.03 and 0.02 μg Se/ml (unpublished results). Selenium in colostrum is normally high, as selenium levels correlate with the amount of milk protein (*Jacobsson et al.* 1965). However, milk protein and selenium content drop drastically as suckling or milking starts.

*Maus et al.* (1980) found that selenium in milk correlates with blood selenium down to a certain limit, below which levels in milk drop comparatively more. We found that if a significant rise in colostral selenium is to be achieved, blood selenium should be at least 0.10 μg Se/ml. In order to achieve an increase in milk selenium on day 7, blood selenium should be at least

0.25 μg Se/ml, a level that is obtained only in sheep given mineral mixture or salt licks containing 25 mg or more of selenium per kg. At these levels, milk may be an important selenium source for the lambs.

Dietary requirement for selenium has been discussed by different investigators. New Zealand workers found that lambs could grow normally at selenium levels in feed of 0.03—0.04 mg/kg (*Andrews et al.* 1976). Other workers have indicated a minimum requirement of 0.06, and *Underwood* (1977) concluded with a general level of requirement at 0.1 mg/kg feed for all animals. It seems to be generally accepted that selenium requirement is closely tied to feeding regime and growth rate. Thus even higher levels have been suggested in recent literature. *Whanger et al.* (1977) reported that 0.1 mg/kg feed is insufficient to prevent NMD in lambs. Based on the effect on glutathione peroxidase activities, *Moksnes & Norheim* (1983) stated that the optimum dietary selenium level for sheep is higher than 0.1 mg/kg. *Harmon* (1983) considered the selenium requirement for fast growing pigs to be 0.3 mg Se/kg feed. *McMurray & Rice* (1982) has looked at trigger factors, and concluded that large amounts of polyunsaturated fatty acids (PUFA), as at spring pasture, are likely to trigger the onset of NMD. They found that the problem arise when alpha-tocopherol concentrations are less than 10 mg/kg and selenium less than 0.2 mg/kg feed.

The most widely used method of administering selenium supplement have been by individual dosing of selenium salts by mouth or by injection, or the deposition of a metal pellet in the rumenoreticulum. Mineral or salt mixtures of different compositions have been tried by *Oh et al.* (1976) and *Ullrey et al.* (1978). Newer methods include the subcutaneous injection of a long acting preparation of barium selenate (*MacPherson & Chalmers* 1984, *Øvernes et al.* 1985) or the deposition in the rumenoreticulum of a slow releasing glass bolus (*Telfer et al.* 1984).

A further way to provide selenium supplement is to increase the selenium content in plants either by foliar application (*Gissel-Nielsen* 1981) or by incorporation of the substance into NPK-fertilizers (*Korkmann* 1984).

At the present time, the Norwegian animal feed regulations prohibit the addition of more than 10 mg Se/kg to supplementary feeds. An increase of the maximum limit to 25 mg Se/kg should provide sufficient protection against disease, without having any toxic effects.

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## SAMMENDRAG

*Virkningen av forskjellige selennivåer i mineralblandinger og saltslikkesteiner på selenstatus hos sau.*

Nivåer på 10, 25 og 40 mg selen (som natriumselenitt) pr kg mineralblanding og saltslikkestein ble utprøvd i inneføringsperioden i sauebesetninger i Numedal, Alvdal og Bærum. Grovfôret i besetningene hadde et innhold av selen under 0,05 mg/kg. Tilskuddet av kraftfôr varierte. Søyenes og lammenes selenstatus ble fulgt ved å måle aktiviteten av glutation peroksydase og nivået av selen i blod fram til beiteslipp. Melkeprøver ble også analysert for innhold av selen. Det ble påvist en meget god korrelasjon ($r = 0,96$) mellom aktiviteten av glutation peroksydase og nivået av selen i blod og disse parametrene var således likeverdige ved måling av selenstatus hos sau. Det var også god korrelasjon mellom blodselen hos lam og deres mødre ($r = 0,85$), og mellom seleninnholdet i råmelk og søyenes ($r = 0,92$) og lammenes ($r = 0,87$) blodselen. I typiske lavselenområder som Numedal og Alvdal er 10 mg selen pr kg i mineralblanding eller saltslikkestein for lite til å dekke selenbehovet hvis det brukes lite kraftfôr. En tilsetning på 25 mg selen pr kg synes å gi tilstrekkelig selendekning hos søyer og lam fram til beiteslipp uten at det medfører noen risiko for overdosering. Effekten var best for mineralblandingene. Saltslikkesteinene gav dessuten større variasjon i resultatene, noe som kan skyldes forskjeller i opptaket av slikkestein.

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