

The effect of Se-amended fertilisers on the Se status of grazing dairy cows

Confidential Report prepared for:
Ravensdown Fertiliser Co-operative Limited

October 2005

Dr ND Grace
AgResearch Limited
Grasslands Research Centre
Private Bag 11008
Palmerston North

Table of Contents

Summary	3
Introduction	3
Study design	3
Results	4
Conclusions.....	5
Introduction	6
Methods and Materials	7
Animals	7
Treatments.....	7
Collection of samples	7
Pasture	7
Blood.....	8
Milk	8
Liver	8
Chemical analyses	8
Results	9
Pasture Se	9
Blood Se	10
Blood glutathione peroxidase activity	11
Serum Se	12
Milk Se	13
Calf blood Se.....	14
Liver Se.....	14
Discussion	15
Conclusion	16
Acknowledgements	16
References	17

Summary

Introduction

Selenium (Se) is an important trace element for dairy cattle. Grazing pastures with Se concentrations <0.03 mg/kg DM results in an inadequate Se intake and poor animal performance. Dairy cattle with blood Se concentrations of <130 nmol/L are Se deficient and will respond to Se supplementation in terms of a 5-10% increase in milk production and an improvement in reproductive performance (reduced services per conception). Blood Se concentration >250 nmol/L reflect an adequate Se status in dairy cattle. The comprehensive fertiliser programme on most dairy farms means that the use of Se-amended fertilisers is an effective and low labour input approach to prevent Se deficiency in dairy cattle.

Selenium-amended fertilisers were developed and evaluated using sheep, and to date there is a dearth of efficacy data for dairy cattle.

Study design

This was designed to evaluate and compare a Ravensdown Selprill Double with a Selcote Ultra prill (Selcote), providing 10 g Se/ha, in terms of changes in blood, serum, liver, and milk Se concentrations, as well as blood glutathione peroxidase activity (GSH-Px) in grazing dairy cows.

The treatments, namely an untreated control, 0.5 kg Ravensdown Selprill Double /ha, and 1 kg Selcote Ultra prill/ha were mixed with 15% potassic superphosphate applied at the rate of 250 kg/ha in early October 2004 on three 6-hectare farmlets. This is Day 1 of the study. After 4 weeks (Day 28) 13 Friesian cows were placed on each farmlet. The small herds were balanced for age, calving date and previous production.

Pasture samples were taken over three 100-150 m transects/farmlet at 2-weekly intervals for the first 3 months, then at 4-5 weekly intervals, for Se determinations.

Likewise, blood samples were taken at 4-6 weekly intervals for blood and serum Se and blood GSH-Px determinations, as well as milk samples at 4-6 weekly intervals for Se determinations. Liver biopsies were taken at Days 1, 99 and 372. The duration of the trial was 372 days. The only feed supplement given to the cows was silage which was made in mid-December (Day 75).

The herd had a marginal Se status; the mean blood Se concentration of the untreated cows was 274 nmol/L, while pasture Se concentrations ranged from 0.02 to 0.04 mg/kg DM. During the latter part of the trial blood Se concentrations were low; 209 nmol/L in untreated control cows.

Results

Untreated pastures contained 0.02-0.04 mg Se/kg DM. The Se concentrations increased to 0.98 and 0.44 mg/kg DM within 10 days after the Se prill application, before decreasing to 0.07 and 0.11 at Day 93, and 0.05 and 0.04 at Day 180 for Ravensdown and Selcote prills, respectively. From Days 180 to 305 pastures treated with Ravensdown prills varied between 0.04 and 0.05 mg Se/kg DM, while pastures treated with Selcote prills slowly increased, being 0.11 mg Se/kg DM at Day 305. Marked changes in the blood Se pools were observed in the cows grazing the Se-treated pastures.

The pre-study blood Se, serum Se and milk Se concentrations were 309, 146 and 59 nmol/L, respectively, while the blood GSH-Px activity was 1.3 kU/L-25°C.

Over the 372-day study the mean blood Se, serum Se, milk Se concentrations and blood of the cows grazing the untreated pasture were 320, 151 and 61 nmo/L, respectively, while the GSH-Px activity was 1.4 kU/L-25°C. For cows grazing the Selcote prill-treated pasture the blood Se concentrations continued to increase, reaching 1364 nmol/L at Day 372. In the case of the cows grazing the Ravensdown prill-treated pasture the blood Se concentrations increased to 1164 nmol/L at Day 99, before gradually decreasing to 740 nmol/L at Day 372.

In general, the changes observed in serum Se and milk Se concentrations, as well as blood GSH-Px activity, were similar to the patterns described for blood Se. The exception was that for cows on the Ravensdown prill-treated pasture, serum and milk Se peaked at Day 64 before decreasing to Day 372.

Likewise, at Day 372 the liver Se concentrations for cows grazing untreated, Selcote prill and Ravensdown prill-treated pastures were 890, 2650 and 1270 nmol/kg fresh tissue, respectively.

Increasing the Se status of the cow markedly increased the Se status of her calf. At calving (Days 314 to 344) the mean blood Se concentrations of the cows on untreated, Selcote prill and Ravensdown prill-treated pastures were 456, 1157 and 1017 nmol/L, respectively. Their respective calf blood Se concentrations were 444, 990 and 1064 nmol/L.

Conclusions

Selenium-amended fertilisers are a very effective approach to increase and maintain an adequate Se status (i.e. blood Se >250 nmol/L) of dairy cows grazing Se-deficient (0.02-0.03 mg Se/kg DM) or low pastures for at least a year. On dairy farms with a comprehensive fertiliser programme Se-amended fertilisers are low labour input, cost effective, safe and efficacious approaches to improving the Se status of the herd.

Introduction

The Se concentration of New Zealand pastures range from 0.005 to 0.07 mg/kg DM (Grant and Sheppard 1983). Pastures containing <0.03 mg Se/kg DM will not provide an adequate Se intake for grazing dairy cows and they will become Se-deficient (Witchel *et al* 1998). An increase in milk production and conception rate to first service, as determined by non return, in response to Se supplementation has been observed in herds with mean blood Se concentrations of <130 nmol/L (Fraser *et al* 1987). Blood Se concentration >250 nmol/L reflect an adequate Se status in dairy cattle. About 30% of New Zealand pastures are considered to have low Se concentrations (Grace 1994). To ensure optimum milk production it is important to determine the Se status of the herd and, if necessary, implement a Se supplementation programme. As dairy farms have a high fertiliser usage, Se-amended fertilisers could be an effective approach to increase the Se status of the herd. The use of Se-amended fertilisers to prevent Se deficiency in sheep is well documented (Watkinson 1983), but there is a dearth of data for dairy cattle.

This study was designed to evaluate the efficacy of Se-amended fertilisers for a dairy herd in terms of changes in blood, serum liver and milk Se concentrations, as well as glutathione peroxidase (GSH-Px) activity.

Methods and Materials

Animals

The study was carried out on the AgResearch Flock House dairy farm, near Bulls in the lower North Island. The property has Se-deficient pastures (0.02-0.03 mg Se/kg DM), and growth responses in beef calves and lambs to Se supplementation have been observed. Thirty nine Friesian cows on the basis of age, calving date, and previous milk production were randomised into 3 groups of 13 animals. Each group of cows was grazed on a 6-hectare farmlet.

All procedures involving the cows were approved by the Crown Research Institutes' Animal Ethics Committee, Palmerston North.

Treatments

The treatments for the dairy cow trial were:

Group 1, control pasture no Se-amended fertiliser applied.

Group 2, Selcote Ultra Se prill applied at the rate of 1.0 kg/ha to provide 10 g Se/ha.

Group 3, Ravensdown Selprill Double applied at the rate of 0.5 kg/ha to provide 10 g Se/ha.

The prills were mixed in 15% potash superphosphate which was applied at the rate of 250 kg/ha in early October 2004. After 4 weeks, during which the rain "washed" the Se-amended fertiliser into the soil, the cows were placed on their respective treatments in November 2004 (Day 28 of the study) and were milked and managed according accepted New Zealand dairy farm practice. Calving occurred during late August/mid September 2005 (Days 320 and 350). In early December surplus pasture on each farmlet was conserved as haylage, which was then fed out during the winter. This meant that a high Se haylage was available to cows grazing pasture treated with Se-amended fertilisers.

Collection of samples

Pasture

Within each farmlet or paddock, pasture samples were collected along three 100-150 m transects prior to the start of the study, and at about 2-weekly intervals for the first three months, and then at about 4-weekly intervals for the rest of the study. The herbage from the transects were then mixed thoroughly and subsampled for Se determinations.

Blood

Prior to the start of the study, and at about 4-6 weekly intervals, blood was collected from the coccygeal vein from 9 monitor cows per group. Two 10 ml vacutainers were used; one without anticoagulant (red top) and one with K₃ EDTA (purple top). The calf was bled from the jugular vein at about 1 day of age. The serum was harvested after centrifuging at 2000 g for 20 minutes.

Milk

Milk was collected at the same time as the cows were bled, except in the case of the first sample taken at start of the lactation, which was 8-15 days after calving.

Liver

Liver biopsies were taken prior to the start of the study and on Days 99 and 372.

Chemical analyses

The Se concentrations in the pasture, blood, serum, liver and milk were determined using the method of Watkinson (1979). Blood GSH-Px activity was assayed using the methods of Paglia and Valentine (1967) and expressed in kU/L determined at a reaction temperature of 25°C.

Results

Pasture Se

The pasture Se concentrations of the untreated pastures ranged from 0.02 to 0.04 mg/kg DM. Applying the Se as prills to provide 10 g Se/ha (Figure 1), in the case of Selcote prill, increased the mean pasture Se concentration (mg/kg DM) to 0.44 at 10 days after application, after which it decreased to 0.06 at 121 days before increasing again to 0.11 at 305 days. The respective responses in mean pasture Se concentrations for the Ravensdown prill were 0.98, 0.06 and 0.04 at Days 10, 121 and 305.

The ranges (n=6) of Se concentration of the haylage made from the surplus spring pasture of the farmlets were 0.01 to 0.02, 0.11 to 0.14, and 0.07 to 0.29 mg Se/DM for the untreated control, Selcote prill and Ravensdown prill-treated pastures, respectively.

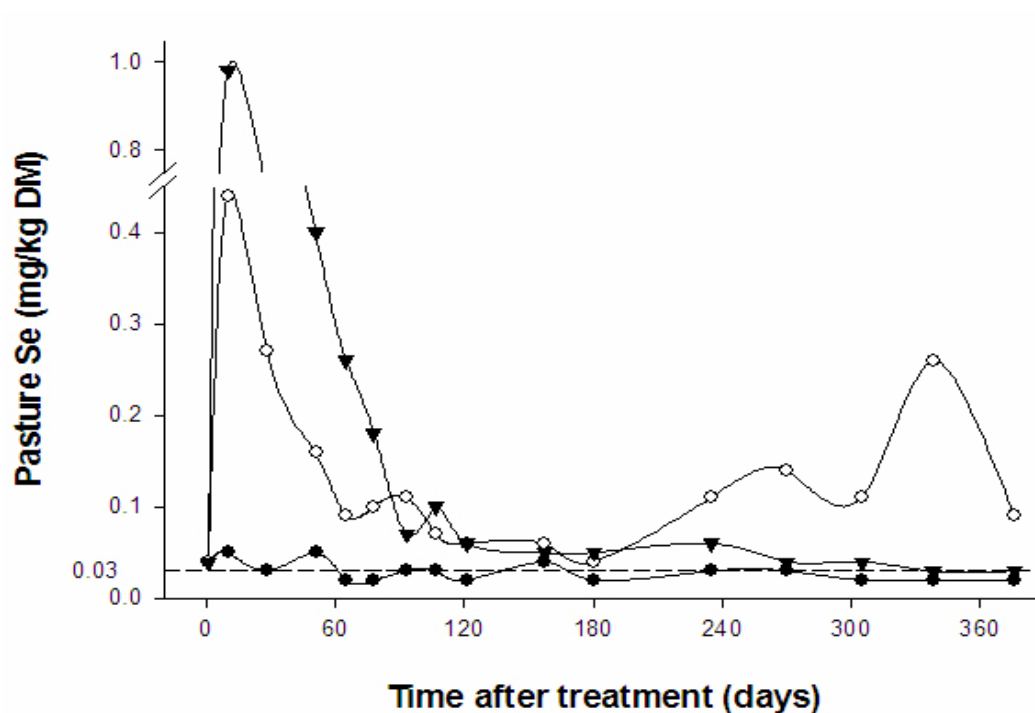


Figure 1. The effect of no Se (♦) and applying 10 g Se/ha as Selcote (□) and Ravensdown (▼) prills on mean pasture Se concentrations of the dairy cow farmlets. Each data point is the mean of pasture sampled from 3 transects (n=3). Pasture Se concentrations greater than 0.03 mg/kgDM will provide an adequate Se intake for grazing dairy cows.

Blood Se

The initial mean blood Se concentration was 320 nmol/L, but in the cows on the untreated pasture this decreased to 220 nmol/L at Day 261, before increasing to 456 nmol/L at Day 328 (Figure 2). In the case of the cows grazing Selcote prill-treated pasture, the blood Se increased to 1364 nmol/L at Day 372, while in cows grazing the Ravensdown prill-treated pasture the blood Se concentration peaked at 1164 nmol/L at Day 99 before decreasing to 740 nmol/L at Day 372.

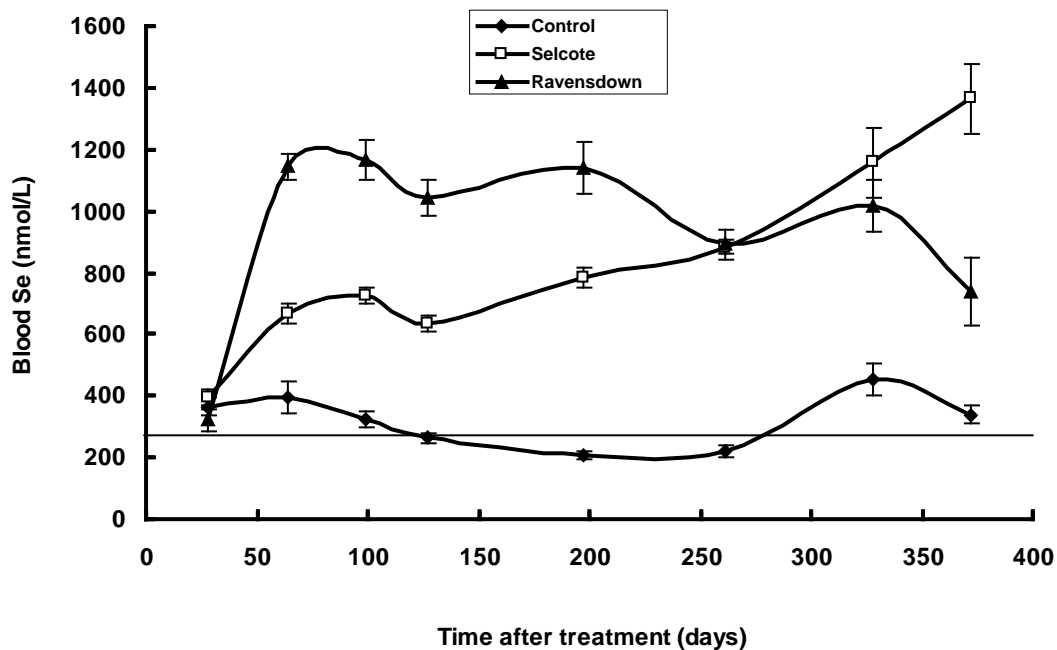


Figure 2. The effect of no Se (◆) and applying 10 g Se/ha as Selcote (□) and Ravensdown (▲) prills on mean (\pm standard error) blood Se concentrations of grazing dairy cows (n=9). Blood Se concentrations of greater than 250 nmol/L are indicative of an adequate Se status.

Blood glutathione peroxidase activity

The initial mean GSH-Px activity was 1.83 kU/L-25°C, but in the cows on the untreated pasture this decreased to 0.58 kU/L-25°C at Day 261, before increasing to 1.86 kU/L-25°C at Day 328 (Figure 3). In the case of the cows grazing Selcote prill-treated pasture the GSH-Px activity increased to 8.58 kU/L-25°C at Day 372, while in cows grazing the Ravensdown prill-treated pasture the GSH-Px activity peaked at 7.54 kU/L-25°C at Day 197, before decreasing to 5.26 kU/L-25°C at Day 372.

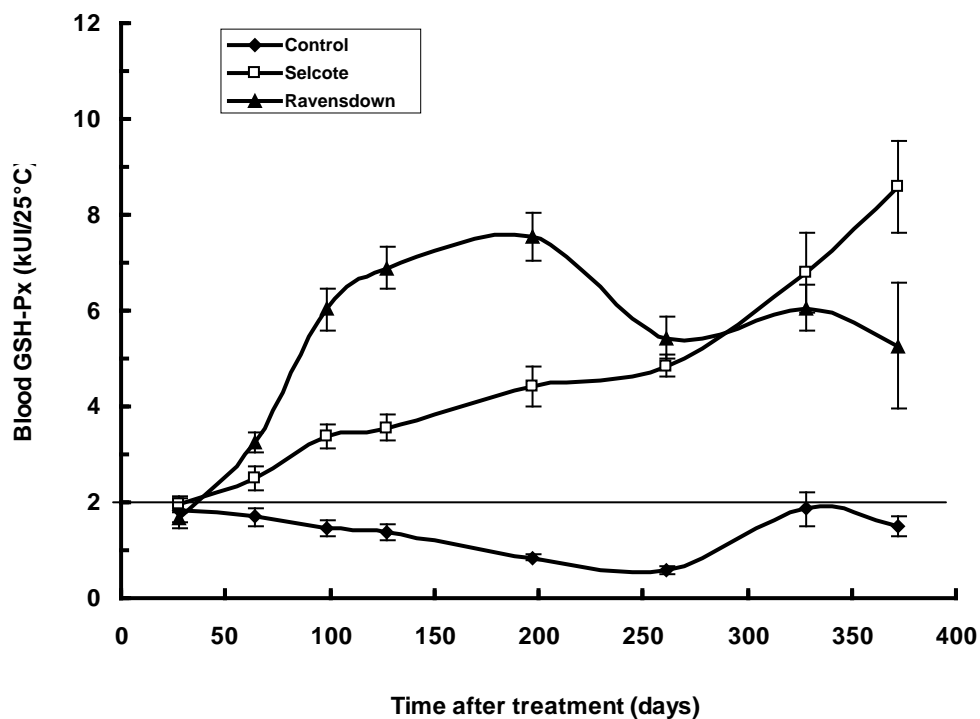


Figure 3. The effect of no Se (♦) and applying 10 g Se/ha as Selcote (□) and Ravensdown (▲) prills on mean (\pm standard error) blood glutathione peroxidase (GSH-Px) activity of grazing dairy cows (n=9).

Blood GSH-Px activity of greater than 2 kU/25°C is indicative of an adequate Se status

Serum Se

The mean initial serum Se concentration was 270 nmol/L, but in cows grazing untreated pasture this decreased to 90 nmol/L at Day 261 (Figure 4). The serum Se concentrations then peaked at 426 and 920 nmol/L at Day 64, before decreasing (particularly in the case of the Ravensdown prill) and then remaining relatively constant for cows grazing the Selcote and Ravensdown prill-treated pastures, respectively.

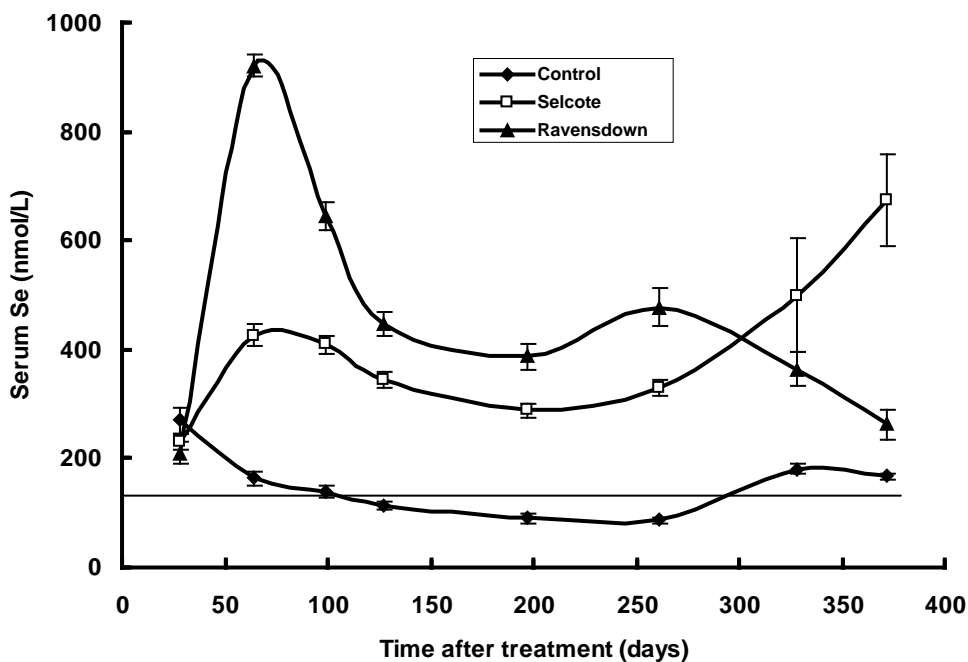


Figure 4. The effect of no Se (♦) and applying 10 g Se/ha as Selcote (□) and Ravensdown (▲) prills on mean (\pm standard error) serum Se concentrations of grazing dairy cows (n=9). Serum Se concentrations of greater than 140 nmol/L are indicative of an adequate Se status.

Milk Se

The mean initial milk Se concentration of the untreated cows was 70 nmol/L, but this then decreased to 58 nmol/L at Day 261, before increasing again to 70 nmol/L at Day 372 (Figure 5). For cows on Se-treated pasture the milk Se concentrations peaked at Day 64, being 136 and 259 nmol/L for cows grazing Selcote and Ravensdown prill-treated pastures, respectively. Milk Se concentrations then decreased to <115 nmol/L at Day 127, and then increased to 260 and 95 nmol/L at Day 372 (early in the second lactation) for cows on the Selcote and Ravensdown prill-treated pastures.

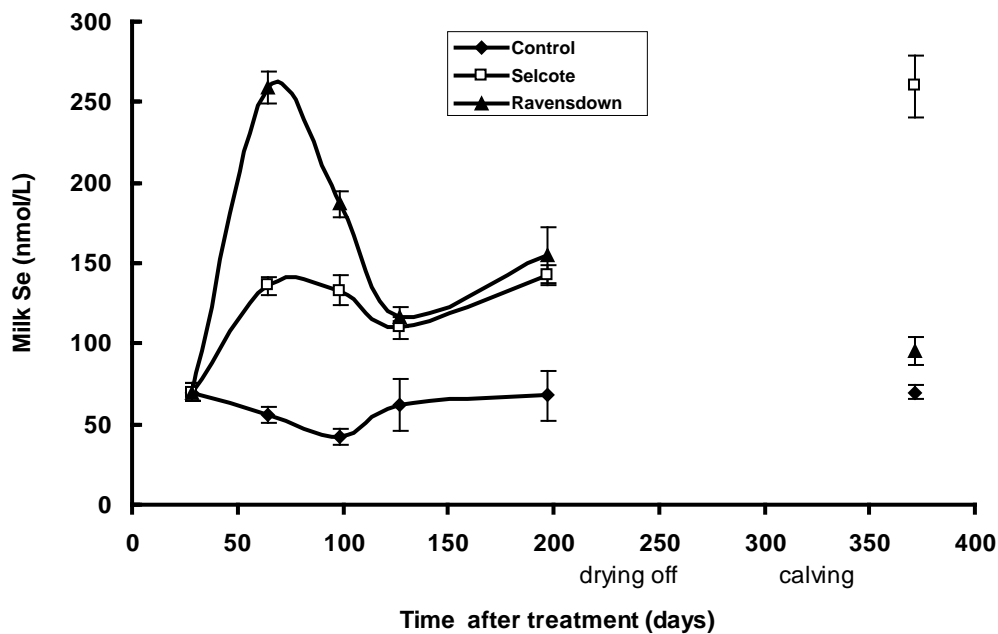


Figure 5. The effect of no Se (♦) and applying 10 g Se/ha as Selcote (□) and Ravensdown (▲) prills on mean (\pm standard error) milk Se concentrations of grazing dairy cows (n=9).

Calf blood Se

The mean blood Se concentrations of calves and cows are shown in Table 1.

Table 1. Effect of grazing untreated and pastures treated with Selcote and Ravensdown prills (10 g Se/ha) on the mean (\pm standard error) blood Se concentrations (nmol/L) of calves and their dams at birth. Calving occurred days 320-350 after treatment.

	Cow	Calf
Control	456 \pm 49.6	444 \pm 37.0
Selecote	1157 \pm 112.6	990 \pm 75.2
Ravensdown	1017 \pm 86.8	1064 \pm 65.7

Liver Se

The mean blood and liver Se concentrations of cows are shown in Table 2.

Table 2. Effect of grazing untreated and pastures treated with Selcote and Ravensdown prills (10 g Se/ha) on the mean (\pm standard error) blood Se concentrations (nmol/L) and liver concentrations (nmol/kg fresh tissue) of cows.

Time on pasture	Day 1		Day 99		Day 372	
	Blood	Liver	Blood	Liver	Blood	Liver
Control	365 \pm 31	510 \pm 34	324 \pm 23	490 \pm 27	334 \pm 34	890 \pm 70
Selecote	282 \pm 28	475 \pm 25	725 \pm 32	1266 \pm 66	1364 \pm 113	2650 \pm 132
Ravensdown	283 \pm 38	490 \pm 28	1192 \pm 64	1714 \pm 58	740 \pm 110	1270 \pm 150

Discussion

Pastures containing <0.03 mg Se/kg DM do not provide adequate Se for dairy cows, and blood Se concentrations of <130 nmol/L are associated with lower milk production and impaired reproductive performance (Fraser *et al* 1987). Further, changes in blood Se concentrations are good indices of the Se status of the dairy cow. The application of Se-amended fertilisers had a marked effect on pasture Se concentrations, increasing them to 0.44-0.98 mg Se/kg DM within 10-28 days, before decreasing to 0.07 mg Se/kg DM after 120 days, and still remaining above 0.04 mg Se/kg DM after 270 days. The peak Se concentration reached, as well as the rate of decline, will be dependent on factors such as the application rate, the form of Se, soil type, and botanical composition. An application rate of 10 g Se/ha is effective in increasing pasture Se concentrations and, thereby, increasing the Se status of livestock without causing any Se toxicity problems. A daily Se intake of 15 mg (dietary concentration of 1mg Se /kg DM) by dairy cows is safe, as this is associated with blood Se concentrations of 1600 nmol/L (Maus *et al* 1980). Blood Se concentrations greater than 13,000 nmol/L are considered to be in the toxic range (Hodges *et al* 1986). In the USA the recommended dietary Se requirement is 0.3 mg Se/kg DM (Gerloff 1992).

The observed profile of changes in Se, that is, the increase and decrease in Se concentrations, in the pasture and blood Se are markedly different (compare Figures 1 and 2). When daily Se intakes exceed Se requirements, cattle are able “store” the Se in body proteins as seleno-amino acids, and this is reflected by the increase and change in blood Se. As there is a rapid increase followed by a marked decrease, within 120 days, in pasture Se concentrations this means that for 90-100 days the daily Se requirements of the grazing livestock are exceeded and Se is stored in the liver and other tissues. However, at low Se intakes blood Se concentrations are now influenced and maintained by tissue Se stores. In this study, blood Se concentrations remained elevated above 250 nmol/L for at least a further 250 days, thus ensuring an adequate Se status in dairy cows for at least 12 months.

The blood has a number of Se pools, namely in the serum and red cell. A large part of the blood Se is found in the red cells, associated with the enzyme glutathione peroxidase, and this is incorporated at the time of red cell formation or erythrocytopoiesis (Thompson *et al* 1981). Therefore, any increase or change in Se intake is reflected by a more rapid increase in serum Se concentrations, followed by a slower increase in blood Se concentrations and blood glutathione peroxidase activity. In this study the serum Se concentration peaked at 36 days (Figure 3) and then it decreased, while the blood Se concentrations reached a peak

much later at 50-60 days and then decreased more slowly (Figure 2 and 6). An exception was in the case of the cows on the Selcote prill-treated pasture, because as the pasture Se concentration of this pasture increased again, the blood Se concentrations of the cows continued to increase, and not decrease as expected (Figures 1 and 2). As changes in blood Se have been related to improved animal performance, the efficacy of Se-amended fertilisers were, therefore, assessed on the basis of changes blood Se concentrations (Fraser *et al* 1987). Blood Se concentrations <250 nmol/L reflect an adequate Se status, as cows with this blood Se concentration do not respond to Se supplementation under New Zealand dairy farm management practices.

Conclusion

This study concluded that the annual application of Se-amended fertilisers, providing 10 g Se/ha, was a low labour input, cost effective, safe and efficacious approach to prevent Se deficiency in grazing dairy cows.

Acknowledgements

I wish to thank Kathryn Baker and Dave Wildermoth for managing the dairy cows. Ravensdown Fertiliser Co-operative Ltd for funding this study.

References

- Fraser AJ, Ryan TJ, Clark RG, Sproule R.** The effect of selenium on milk production and fertility in dairy cattle on the Rangitaiki Plains. *Proceedings of the 4th Animal Science Congress of the Asian-Australian Association of Animal Production Societies*. Pp 427, 1987.
- Gerloff BJ.** Effect of selenium supplementation on dairy cattle. *Journal of Animal Science* 70, 3934-40, 1992.
- Grace ND.** Managing Trace Element Deficiencies. AgResearch, Palmerston North, 1994.
- Grace ND, Knowles SO.** The use of blood selenium concentrations to predict growth responses to selenium supplementation in lambs. *New Zealand Veterinary Journal* 50, 163-5, 2002.
- Grant AB, Sheppard AD.** Selenium in New Zealand pastures. *New Zealand Veterinary Journal* 31, 131-6, 1983.
- Hodges RT, Read DH, Brooks HV.** Specimens for Veterinary Laboratory Diagnosis. Animal Health Division, MAF, New Zealand. Pp 212, 1986.
- Maus, RW, Martz FA, Belvea RL, Weiss MF.** Relationship of dietary selenium to selenium in plasma and milk from dairy cows. *Journal of Dairy Science* 63, 532-7, 1980.
- Paglia DE, Valentine WN.** Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *Journal of Laboratory and Clinical Medicine* 70, 158-9, 1967.
- Thompson KG, Fraser AJ, Harrop BM, Kirk JA, Bullians J, Cordes DO.** Glutathione peroxidase activity and selenium concentration in bovine blood and liver as indicators of dietary selenium intake. *New Zealand Veterinary Journal* 29, 3-6, 1981.
- Watkinson JH.** Semi-automated fluorometric determination on nanogram quantities of selenium in biological materials. *Analytica Chimica Acta* 105, 319-25. 1979
- Watkinson JH.** Prevention of selenium deficiency in grazing animals by annual topdressing of pasture with sodium selenate. *New Zealand Veterinary Journal* 31, 78-85, 1983.
- Witchel JJ.** A review of selenium deficiency in grazing ruminants. Part 2: Towards a more rational approach to diagnosis and prevention. *New Zealand Veterinary Journal* 46, 54-8, 1998.