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## Effect of Se-amended fertilisers on the Se status of grazing dairy cattle

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### ABSTRACT

Selenium (Se) is an important trace element for dairy cattle and deficiencies (0.03 mg Se/kg DM) are associated with lowered milk yields and impaired fertility in grazing cows. As many dairy farms have a comprehensive fertiliser programme the use of Se-amended fertilisers (prills) could be an effective approach to prevent Se deficiency. Three farmlets (6-7 ha) were established and grazed with 13 cows/farmlet balanced for age, calving date and production. The treatments were 1) untreated control, 2) Sel Se prill (Selecote Ultra), and 3) Rav Se prill (Ravensdown Selprill Double). The prills were mixed with 15% potassic superphosphate and applied at 250 kg/ha, in early October, to provide 10 g Se/ha. This is Day 1 of the study. The cows were allocated to treatments 4 weeks later and changes in pasture Se, blood Se, serum Se, milk Se concentrations and blood glutathione peroxidase activity were monitored over the next 12 months. Untreated pasture Se concentrations ranged from 0.03 to 0.04 mg/kg DM, while pastures treated with prills had pasture Se concentrations > 0.5 mg/kg DM within 14 days after treatment, before decreasing to 0.06 mg/kg DM after 121 days, after which they ranged between 0.06 and 0.26 mg Se/kg DM for the Sel prill, and between 0.06 and 0.03 mg/kg DM for the Rav Se prill over the next 251 days. The mean ( $\pm$  SE) blood Se of cows grazing the control pasture was  $280 \pm 30$  nmol/L, while in cows grazing pasture treated with the Sel Se prill blood Se concentrations increased to  $1364 \pm 112$  nmol/L after 372 days. In the case of cows grazing pasture treated with Rav Se prill, blood Se concentrations increased to  $1192 \pm 66$  nmol/L within 60 days, before decreasing slowly to  $740 \pm 65$  nmol/L at 372 days. Blood glutathione peroxidase activity followed a similar pattern to the blood Se. The mean serum Se concentrations of the untreated cows was 151 nmol/L, while in animals on the Sel Se prill-treated pasture they reached a small peak at Day 64, followed by a decrease and then continued to increase to 675 nmol/L at Day 372. In contrast, for cows on the Rav Se prill treatment the serum Se concentrations reached a peak of 920 nmol/L at Day 64, and then decreased to 263 nmol/L at Day 372. Milk Se followed a similar pattern to serum Se. Selenium-amended fertilisers were found to be effective in raising the pasture Se concentrations for 4 or 12 months, depending on Se prill composition, which then in turn increased blood Se concentrations of dairy cows for at least 12 months.

**Keywords:** dairy cows; Se-amended fertiliser; blood Se; serum Se; blood glutathione peroxidase; milk Se.

### INTRODUCTION

The Se concentration of New Zealand pastures range from 0.005 to 0.07 mg/kg DM (Grant & Sheppard, 1983). Pastures containing < 0.03 mg Se/kg DM will not provide an adequate Se intake for grazing dairy cows and they are likely to become Se-deficient (Wichtel, 1998). An increase in milk production and conception rate to first service 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 an effective 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 dairy cattle in terms of changes in blood, serum and milk Se concentrations, as well as blood glutathione peroxidase (GSH-Px) activity.

### MATERIALS AND METHODS

#### 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 lambs to Se supplementation have been observed (Grace & Knowles, 2003). Thirty nine Friesian cows were randomised into three groups of 13 animals on the basis of age, calving date, and previous milk production. Each

group of cows was grazed on a 6-7 ha 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: Sel Se prill (Selcote Ultra (containing barium and sodium selenate); Nufarm Auckland, New Zealand) applied at the rate of 1.0 kg/ha to provide 10 g Se/ha.

Group 3: Rav Se prill (Ravensdown Selprill Double (containing sodium selenate); Ravensdown Fertiliser Co-operative Ltd, Christchurch, New Zealand) applied at the rate of 0.5 kg/ha to provide 10 g Se/ha.

The prills were mixed in 15% potassic superphosphate which was applied at the rate of 250 kg/ha in early October 2004 (Day 1 of the study). After 4 weeks the cows were placed on their respective treatments in November 2004 (Day 28), and were milked and managed according to accepted New Zealand dairy farm practice. Calving occurred during late August/mid September 2005 (Days 320 and 350). In early December 2004 (Day 58) surplus pasture on each farmlet was conserved as silage, which was then fed out during the winter to the cows in their respective groups. 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, on three paddocks, pasture samples were collected along three 100-150 m transects prior to the start of the study, at 13-16-day intervals for the first three months, and then at 26-30-day intervals for the rest of the study. The herbage from the three transects, within a farmlet, were then mixed thoroughly and a bulked sample, on a weight basis, was provided for every treatment at each time of sampling for Se determinations.

#### Blood

Prior to the start of the study, and at 4-6-weekly intervals thereafter, 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 K<sub>3</sub> EDTA (purple top). The serum was harvested after centrifuging at 2000 g for 20 mins.

#### Milk

Milk samples were collected at the same time as the cows were bled.

### 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 & Valentine (1967) and expressed in kU/L determined at a reaction temperature of 25°C.

### Statistics

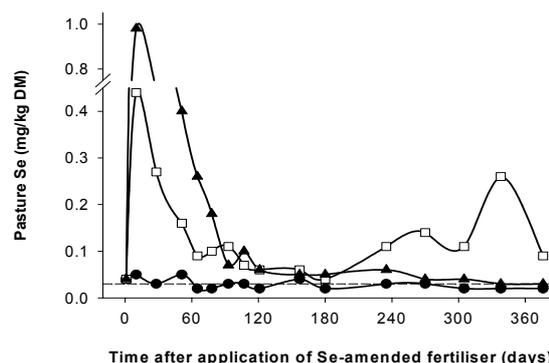
Significant differences between treatments for tissue Se concentrations were determined by the General Linear Model (GLM) procedures of SAS (version 8.02, SAS Inc., Cary, NC, USA) using the PDIF option of the LSMEANS test for difference from control, with Dunnett's adjustment for one-tailed comparisons. Treatment effects and differences between means were considered significant when  $P < 0.05$ . Unless otherwise indicated, values are reported as means  $\pm$  standard error.

## RESULTS

### Pasture Se

The 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 the Sel Se prill, increased the mean pasture Se concentration (mg/kg DM) to 0.44 at Day 14; it then decreased to 0.06 at Day 121 before increasing again to 0.26 at Day 338. The mean pasture Se concentrations for the Rav Se prill were 0.98, 0.06, 0.04 and 0.03 at Days 10, 121, 305 and 376, respectively. When compared with untreated pasture the Se prills significantly ( $P < 0.01$ ) increased pasture Se concentrations for at least 300 days.

**FIGURE 1:** The effect of applying no Se (●), 10 g Se/ha as Sel (□) and Rav (▲) prills on mean pasture Se concentrations of the dairy cow farmlets. Each data point is the value of a bulked sample of pasture collected from three transects per farmlet. Pasture Se concentrations > 0.03 mg/kg DM will provide an adequate Se intake for grazing dairy cows.



The ranges ( $n = 6$ ) of Se concentration of the silage made from the surplus spring pasture of the farmlets were  $0.017 \pm 0.002$ ,  $0.14 \pm 0.09$ , and  $0.23 \pm 0.10$  mg Se/kg DM for the untreated control, Sel prill and Rav prill-treated pastures, respectively.

### 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 by Day 261, before increasing to 456 nmol/L at Day 328 (Figure 2a). In the case of the cows grazing Sel Se prill-treated pasture, the blood Se increased to 1364 nmol/L at Day 372, while in cows grazing the Rav Se prill-treated pasture the blood Se concentration peaked at 1164 nmol/L at Day 99 before decreasing to 740 nmol/L at Day 372. Cows grazing Se-treated pastures had significantly higher ( $P < 0.001$ ) blood Se concentrations when compared with animals on untreated pastures at each sampling period throughout the study.

### Blood glutathione peroxidase activity

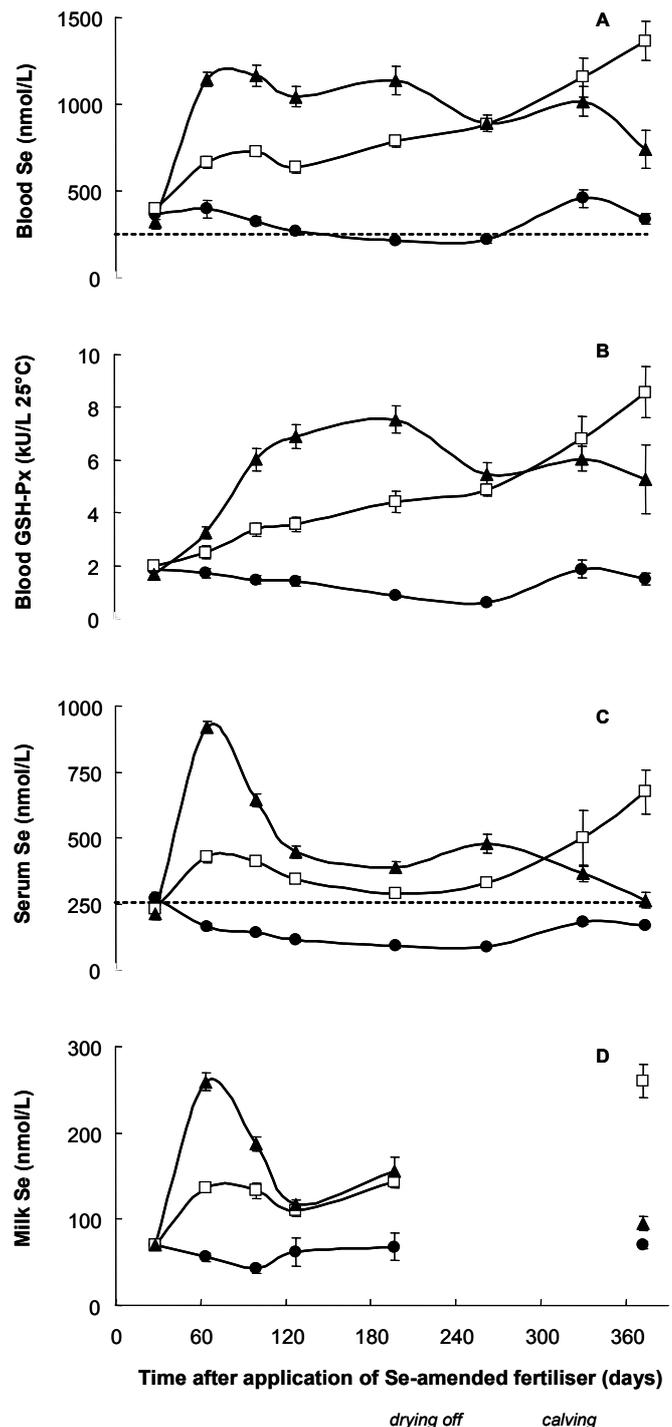
The initial mean GSH-Px activity was 1.83 kU/L-25°C, but in the cows on the untreated pasture this decreased to 0.58 kU/L-25°C at Day 261, before increasing to 1.86 kU/L-25°C at Day 328 (Figure 2b). In the case of the cows grazing Sel Se prill treated pasture the GSH-Px activity increased to 8.58 kU/L-25°C at Day 372, while in cows grazing the Rav Se 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. Cows grazing Se-treated pastures had significantly higher ( $P < 0.001$ ) blood GSH-Px activity when compared with animals on untreated pastures at each sampling period throughout the study.

### 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 2c). For the cows on the Sel Se prill treatment the serum Se concentrations continued to increase during the study, reaching 675 nmol/L at Day 372. In contrast, for cows on the Rav Se prill treatment the serum Se concentrations peaked at 920 nmol/L at Day 64, before decreasing to 263 nmol/L at Day 372.

Cows grazing Se-treated pastures had significantly higher ( $P < 0.001$ ) serum Se concentrations, when compared with animals on untreated pastures at each sampling period up to Day 328.

**FIGURE 2:** The effect of applying no Se (●), 10 g Se/ha as Sel (□) and Rav (▲) prills on mean (A) blood Se concentrations, (B) blood glutathione peroxidase activity, (C) serum Se concentrations, and (D) milk Se concentrations of grazing dairy cows ( $n = 9$ ). Vertical bars represent standard errors. Blood Se concentrations of  $> 250$  nmol/L are indicative of an adequate Se status. Blood GSH-Px activities of  $> 2$  kU/L-25°C are indicative of an adequate Se status. Serum Se concentrations of  $> 140$  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 2d). For cows on Se-treated pasture the milk Se concentrations follow a similar pattern to that observed for serum Se. Milk Se concentrations were 260 and 95 nmol/L at Day 372 (early in the second lactation) for cows on the Sel Se and Rav Se prill treated pastures, respectively. Cows grazing Se-treated pastures had significantly higher ( $P < 0.001$ ) milk Se concentrations, when compared with animals on untreated pastures at each sampling period up to Day 197.

### DISCUSSION

Pastures containing  $< 0.03$  mg Se/kg DM do not provide an adequate Se intake 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; Wichtel, 1998). As changes in blood Se concentrations are a good measure of the Se status of the dairy cow the efficacy of the Se-amended fertilisers was evaluated in terms of changes of blood Se parameters (Wichtel, 1998).

The application of Se-amended fertilisers had a marked effect on pasture Se concentrations, increasing them to 0.5-0.98 mg Se/kg DM within 14 days, before decreasing to 0.06 mg/kg DM after 121 days, after which they ranged between 0.04 and 0.26 mg Se/kg DM for a further 261 days. The pattern of pasture Se uptake was different for the Sel Se and Rav Se prill-treated pastures (Figure 1). The peaks of Se concentration reached, as well as the rate of decline, will be dependent on factors such as the application rate, the form of Se in the product, prill formulation, 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 (i.e. dietary concentration of about 0.75 mg Se/kg DM) by dairy cows is safe, as this was associated with blood Se concentrations of 1600 nmol/L (Maus *et al.*, 1980). As blood Se concentrations greater than 13,000 nmol/L are considered to be in the toxic range, this study showed a safety factor of at least 9, as the highest blood Se observed was 1364 nmol/L (Hodges *et al.*, 1986).

The observed profiles of change in Se, that is, the increase and decrease in Se concentrations, of 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 in blood Se concentrations. However, during periods of low Se intakes the blood Se concentrations are influenced and maintained by tissue Se stores. This is well illustrated by cows on the Rav Se prill-treated pasture, while the relationship between pasture Se and blood Se concentrations for cows on the Sel Se prill-treated pasture is more complex, because the pasture Se concentrations peaked at both Days 14 and 338, due to the readily available sodium selenate and then the more slowly available barium selenate content.

The blood has a number of Se pools, namely in the serum and red cells. 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 erythropoiesis (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. For example, it was observed in cows grazing the Rav Se prill-treated pasture that the serum Se concentration peaked at 36 days (Figure 2) and then decreased, while the blood Se concentrations reached a peak much later at 50-60 days and then decreased more slowly (Figure 2). The pattern of changes in the serum and blood Se concentrations of the cows grazing the Sel Se prill-treated pastures was more complex because of the marked variation in Se intake as pasture Se concentrations peaked twice, for the reason given above.

In this study, as blood Se concentrations remained elevated above 250 nmol/L for at least 372 days in cows grazing pastures treated with Se, both prill types in the Se-amended fertilisers are therefore a very effective approach to prevent Se deficiency in dairy cows for at least 12 months.

### ACKNOWLEDGEMENTS

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