The Effect of seed pelletization with selenite on the yield and selenium uptake of ryegrass cultivars

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Abstract
A greenhouse experiment was conducted to assess the effect of seed pelletization with increasing selenite supply levels (from 0 to 60 g ha^{-1}) on the yield, uptake and distribution of Se in three ryegrass cultivars (Aries, Nui and Quartet) growing in an Andisol never amended with Se fertilizers. For the ryegrass cultivars, the yield of shoots (first and second cut) and roots was not affected by the application of selenite directly to the seeds at rates up to 60 g Se ha^{-1}. Selenite-pelleted seeds at rates from 5 to 60 g Se ha^{-1} progressively increased the Se concentration in shoots and roots, and most of Se was accumulated in the roots. Furthermore, Se dosages above 10 g ha^{-1} increased the shoot Se concentration in two cuts to proper levels according to the minimum dietary requirement of cattle. Thus, the use of selenite-pelleted ryegrass seeds appears to be a strategic tool to increase the forage Se content under field conditions.

Key Words
Seed pelletization, selenite, selenium fertilization, ryegrass, Andisol, pasture

Introduction
Since 1973, selenium (Se) is well recognized as an essential trace element for animal nutrition because it is an integral part of several proteins including the antioxidant enzyme glutathione peroxidase and other Se-containing enzymes such as iodothyronine deiodinases, thioredoxin reductase and selenoprotein W (Pallud *et al.,* 1997; Birringer *et al.,* 2002).
Selenium deficiency is a well-known issue in some areas of North America, New Zealand, Australia, China and Finland (Gissel-Nielsen *et al.,* 1984; Gupta and Gupta, 2000; Hartikainen, 2005). In Chile, over 60% of livestock production is concentrated in grasslands of the South region, and low Se concentration in pastures have been associated with metabolic disorders in grazing cattle (Wittwer *et al.,* 2002), which denote the need for Se supplementation. The application of Se-fertilizers has proven to be an effective way to raise the Se concentration of forages in Se-deficient areas (Whelan and Barrow, 1994; Oldfield, 1998; Hartikainen, 2005). However, in the Chilean market there are not Se-fertilizers, and the nutritional diseases associated with the low Se intake support the need to develop technologies for Se application in deficient pasture areas. In this sense, the pelletization of seeds with selenite has been successfully employed by Mora *et al.* (2008) to increase the Se content in white clover (*Trifolium repens*). However, this plant species is very sensitive to Se toxicity, which could severely decrease its persistence in grass-legume permanent pastures leading to a reduction of the nutritional quality of forage.
On the other hand, ryegrass (*Lolium perenne*) is one of the most important plant species in terms of its contribution to the botanical composition of permanent pastures of Southern Chile, and our previous studies have shown non-detrimental effect of selenite on ryegrass yield at low Se addition levels (Cartes *et al.,* 2005; 2006). These facts suggest that the use of selenite-pelleted ryegrass seeds needs to be evaluated as a strategic tool to increase the Se content in Se-deficient pastures. In addition, plant species differ widely in their ability to accumulate Se (Rani *et al.,* 2005), and differences in Se uptake have also been observed among different ryegrass cultivars growing in a Se deficient Andisol (Cartes, 2005), which denote the need to evaluate the dynamics of Se accumulation at the cultivar level. The aim of this study was to evaluate the effect of seed pelletization with increasing selenite supply levels on the yield, uptake and distribution of Se in different ryegrass cultivars growing in an Andisol under greenhouse conditions.

Methods
Ryegrass (*Lolium perenne*) plants of three cultivars (Aries, Nui and Quartet) were cultivated in a greenhouse experiment with an Andisol (Freire Series of Southern Chile) never amended with Se-fertilizers. The soil had a pH of 5.65 (1 soil:2.5 H_2O), an extractable P content of 20 mg kg^{-1} (Olsen bicarbonate extract, analyzed by the Murphy and Riley (1962) method), an extractable S content of 15 mg kg^{-1} ($Ca(H_2PO_4)$ extract,

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determined by turbidimetry according to the Tabatabai (1982) method), an organic matter content of 15% (modified Walkley–Black procedure), and the following exchangeable cations (cmol(+) kg^{-1}): K 0.95, Na 0.15, Ca 10.2, Mg 1.41 (extracted with 1M NH_4-acetate solution at pH 7.0) and Al 0.06 (extracted with 1M KCl solution).

For the pot experiment, 2.2 kg soil samples were weighed, and each soil sample was fertilized with 200 mg P kg^{-1} (as Triple superphosphate) and with 100 mg S kg^{-1} (as potassium magnesium sulfate ($K_2SO_4x2MgSO_4$ salt), Sulpomag). Before sowing, the seeds of each ryegrass cultivar were pelleted with a mixture of adhesive solution, sodium selenite ($Na_2SeO_3x5H_2O$, Merck reagent) and dolomite as coating material. Selenium was supplied to the seeds at increasing rates equivalent to field applications of 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60 g ha^{-1}. The doses of Se were calculated according to the ryegrass seedling rate (25 kg ha^{-1}) commonly used in permanent pastures in Southern Chile. One hundred and fifty pelleted seeds of each ryegrass cultivar were sown, and after germination plants were thinned to 80 seedlings per pot. For each Se treatment, three pots were used as replicates.

During the growth period, the plants were watered daily with distilled water. Two cuts of shoot biomass were harvested at 30 cm plant height, and at the end of the experiment the roots were also collected. Shoots and roots were dried at 65ºC for 48 h to determine dry weight (DW) and Se concentration. For Se analysis, the dried plant samples were digested in an acid mixture (HNO_3, $HClO_4$ and H_2SO_4) as described by Kumpulainen *et al*. (1983), and the Se concentration was measured by Hydride Generation Atomic Absorption Spectrophotometry (HGAAS).

Yield data were subjected to analysis of variance (ANOVA), and significantly different means were separated with the Tukey's test ($p \leq 0.05$). The effect of applied Se on the plant Se concentration was investigated by polynomial regression analysis ($p \leq 0.05$).

Results

In terms of plant yield, we did not find significant interactions between the different ryegrass cultivars and the Se dosages ($p \leq 0.05$). Likewise, non significant differences in the dry weight (DW) of shoots (first and second cut) and roots of Aries, Nui and Quartet were observed by effect of Se addition at rates between 0 and 60 g Se ha^{-1}. These results were expected since in a preliminary experiment we did not detect any change in the yield of ryegrass cv. Aries by effect of seed treatment with either selenite or selenate at low rates of Se application (Cartes, 2005). In the case of shoots, we only found statistically significant differences between cultivars (for the same cut) and between cuts (for the same cultivar), irrespective of the Se addition. Figure 1 shows that the highest average shoot yield occurred in Quartet at the first cut and in Aries at the second cut, and for each ryegrass cultivar the averaged shoot yield was increased by about 38% (Aries), 25% (Nui) and 16% (Quartet) at the second cut. Furthermore, the averaged root dry weight differed significantly between cultivars, and the greatest root yield was observed in ryegrass Aries.

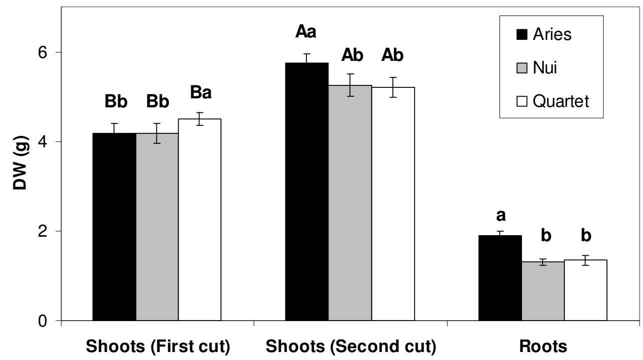

Figure 1. Dry weight (DW) of shoots (first and second cut) and roots of three ryegrass cultivars subjected to Se treatment at rates between 0 and 60 g ha^{-1} applied to the seeds. Different upper case letters show significant differences ($p \leq 0.05$) between the first cut and the second cut for the same cultivar. Different lower case letters indicate statistically significant differences ($p \leq 0.05$) between cultivars for shoots (first or second cut) and for roots, according to the Tukey's test.

Previous studies have demonstrated that the use of Se-fertilizers did not reduce the yield of forages and increased the Se concentration in plant tissues at proper levels according with the requirements for animal nutrition (Whelan and Barrow, 1994; Hartikainen, 2005). In this study, selenite applied directly to the seeds at supply levels from 5 to 60 g Se ha^{-1} steadily increased the shoot Se concentration at the first and the second cut of ryegrass cv. Aries, Nui and Quartet (Figure 2a,b; $p \leq 0.05$). At equal rates of Se application, no differences in shoot Se concentration were observed between the different ryegrass cultivars. The only exception was the Se dosage of 60 g Se ha^{-1}, which raised the Se concentration in Quartet by at least 22% (first cut) and 25% (second cut) with respect to Aries or Nui. Selenium accumulation was reduced by about 27% in the shoots of the three cultivars in the second cut. Nevertheless, when Se was applied at rates above 10 g ha^{-1}, its concentration in the second cut remained above 100 µg kg^{-1}, which is the minimum dietary level recommended for ruminant nutrition (NRC, 2000).

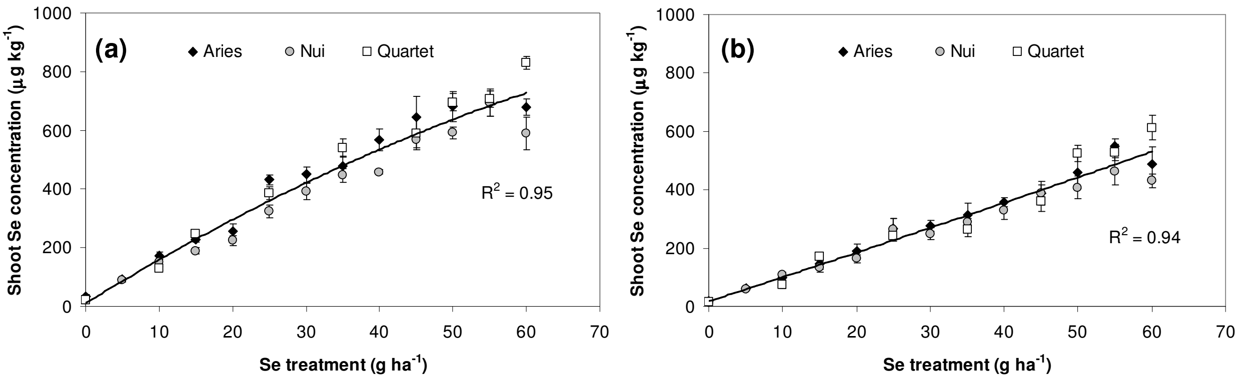

Figure 2. Shoot Se concentration at the first cut (a) and the second cut (b) of three ryegrass cultivars subjected to Se treatment at rates between 0 and 60 g ha^{-1} applied to the seeds.

From our results, it can also be seen that most of Se taken up by plants was accumulated in the roots (Figure 3). Interestingly, there were noticeable differences in the root Se concentration between the ryegrass cultivars. Even though similarities in root Se concentration were found between Aries and Nui, Quartet accumulated more Se in their roots than the other ryegrass cultivars, at Se supply levels above 35 g ha^{-1}. For example, when 60 g Se ha^{-1} were applied, the root Se concentration was at least 60% higher in Quartet than in Aries or Nui.

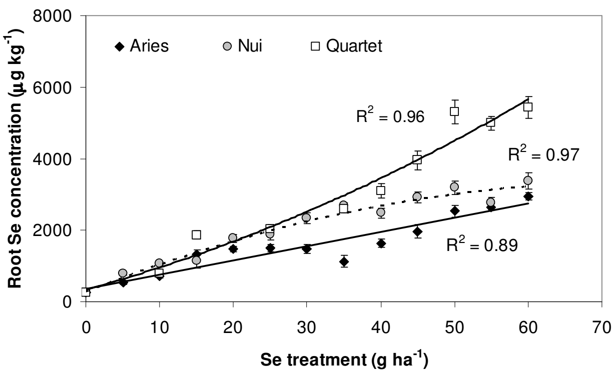

Figure 3. Root Se concentration of three ryegrass cultivars subjected to Se treatment at rates between 0 and 60 g ha^{-1} applied to the seeds.

The differences in root Se concentration (Figure 3) and yield (Figure 1) between cultivars rendered differential efficiencies in the total uptake of Se by plants: whereas Quartet accumulated an average of 1.79% of the total Se supplied to the plants, in Aries and Nui the averaged efficiency lowered to 1.68% and 1.54%, respectively. The low amount of Se taken up by ryegrass plants was as expected because selenite is strongly adsorbed in Andisols (Barrow *et al.,* 2005). Nevertheless, selenite continues reacting over a long period with the soil constituents (Barrow and Whelan, 1989), representing a long term source of available Se in the rhizosphere.

Conclusion

Seed pelletization with selenite at dosage between 15 and 60 g Se ha^{-1} increased the shoot Se content in two cuts of ryegrass Aries, Nui and Quartet above the minimum Se dietary requirement of beef and dairy cattle, without any negative effect on the plant yield. Further studies are required to test the use of selenite-pelleted ryegrass seeds as a strategic tool to increase the Se concentration in Se-deficient pasture areas.

Acknowledgments

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