Supplementation of selenium to sheep grazing kikuyu or ryegrass: II. Effect on selenium concentration in the grass and body tissues

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The investigation entailed four trials in which selenium (Se) was supplemented to lambs grazing kikuyu (*Pennisetum clandestinum*) pastures during two summer periods or Italian ryegrass (*Lolium multiflorum*) pastures during two winter periods. Two methods of supplementing the lambs were tested, via fertilizing the pasture with Se and/or injecting a long-acting parenteral Se supplement, and compared with the unsupplemented controls. Ryegrass was more effective ($P < 0.01$) in accumulating Se when fertilized than kikuyu where the response was so low that the lambs could not meet their Se requirements through the supplemented kikuyu. The tissue Se concentrations of the lambs on Se fertilized ryegrass were higher ($P < 0.001$) than those in the controls, for example hepatic Se concentrations of the two control groups were 124 and 137 ng/g dry matter (DM) compared to 965 and 848 ng Se/g DM in the respective groups consuming the Se fertilized ryegrass. Parenteral supplementation was effective in supplying Se requirements of the lambs. However, lambs that lost weight during the second season on kikuyu had higher ($P < 0.01$) tissue Se concentrations than the parenterally supplemented groups during the other trials. Selenium tended to accumulate in a non-linear and decreasing rate with an increase in level of Se intake. It was concluded that with the present recommendations Se fertilization of kikuyu cannot be recommended as a method of supplementing Se to the grazing animal.

Keywords: Selenium, sheep, kikuyu, ryegrass, parenteral, fertilized, toxicity

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Introduction

Different methods of supplementing selenium (Se) to livestock have been practised world-wide with varying degrees of success. Supplying Se in a lick can be a problem because of the wide fluctuation in lick intake among sheep (Money *et al.*, 1986). Direct administration of Se by dosing or injecting gave satisfactory results especially with slow-release long-acting products (Langlands *et al.*, 1990; Van Ryssen *et al.*, 1992). However, these methods are expensive and require the handling of the animal (Whelan *et al.*, 1994). Topdressing pastures with Se, usually mixed with other fertilizers, has proven to be effective (Watkinson, 1983). That is the almost exclusive means of Se supplementation in Finland (Gissel-Nielsen, 1993) and a common practice in livestock production in New Zealand (Watkinson, 1983) and in the high rainfall areas of Australia (Whelan *et al.*, 1994). A slow releasing Se fertilizer product has been introduced to South Africa, but little information has been published on its effectiveness under local conditions.

A risk of an inadvertent oversupply of Se through supplying Se through two or more methods does exist (Van Ryssen *et al.*, 1992). Selenium has a narrow margin between a therapeutic and a toxic dose (Allen & Mallison, 1984; Archer & Judson, 1994), emphasized by reports that the maximum safe dietary concentration in many situations is closer to 1 mg/kg dry matter (DM) (Hakkarainen, 1993) than to the generally accepted range of 3 to 5 mg/kg DM (Mayland, 1994).

In this report of the investigation we examined the response to Se fertilization of two grass species widely cultivated in KwaZulu-Natal, namely kikuyu (*Pennisetum clandestinum*) and Italian ryegrass (*Lolium multiflorum* cv. Midmar), and the effect of two methods of Se supplementation of sheep on Se concentrations in their tissues.

Materials and methods

Animals and treatments

Four separate grazing trials were conducted with SA Mutton Merino weaner lambs. In two, lambs grazed dryland kikuyu pastures during two summer periods (K1 and K2) and in two, irrigated annual Italian ryegrass pastures for two winter periods (R1 and R2). During each trial 20 lambs (24 were used in K2) were divided randomly into four groups according to a 2 × 2 factorial experimental design. The treatments were: an unsupplemented control; the pasture fertilized before the onset of the trial with 1 kg/ha of a slow releasing Se fertilizer (Selcote Ultra, ICI Crop Care, Wellington, New Zealand); the lambs injected subcutaneously with slow-releasing Se product (0.5 ml/25 kg body mass of barium selenate suspended in a viscous petroleum base, containing 50 mg Se/ml; Deposel, Rycovet Ltd., Glasgow, Scotland) at the onset of each trial; and the combined supplementation of fertilized pasture and Se injection. The sheep were slaughtered at the end of each grazing period: after 172 days in K1, after 132 days in K2; after 80 days in R1 and after 98 days in R2.

The investigation was conducted at the Cedara Agricultural Research Station, situated in the KwaZulu-Natal Midlands at an altitude of 1067 m and with an average annual rainfall 885 ± 142 mm, predominantly in summer. The kikuyu was grown on a Hutton/Devon soil and the ryegrass in a vleiland on a Katspruit soil. The lambs stayed in their respective grazing paddocks for the duration of each trial. The treatment groups receiving the Se fertilizer grazed together and those without Se fertilizer grazed an adjacent paddock. Stocking rates per paddock were applied according to the prescribed rates for the experimental farm (Van Ryssen *et al.*, 1999). A different paddock was fertilized each season, though the same unfertilized paddocks were used in the two seasons. The sheep had free access to a mineral/salt (without Se) lick and water. The chemical properties of the soils were determined before the onset of each grazing season by the Cedara Fertilizer Advisory Service and the pastures were fertilized according to their recommendation. One application of ammonium sulphate in October and two applications of limestone ammonium nitrate (LAN – 28%) during November and February were applied to the kikuyu pasture, resulting in a 250 kg N/ha application rate for the season. The ryegrass pasture was fertilized at 350 kg N/ha/season. The N was applied in the form of LAN (28%) in six dressings of 58 kg N/ha/dressing.

Sampling and chemical analyses

A representative sample of the grass per paddock was collected approximately once a month, dried at 80°C and stored pending Se analysis. Sampling of the Se fertilized grass commenced approximately three weeks after Se application. Pasture height was measured monthly with a pasture disc meter to ensure the correct stocking rates. At approximately monthly intervals during each trial, blood samples were collected by jugular venipuncture in evacuated tubes containing lithium heparin as anticoagulant. The fresh livers, kidneys and carcasses were weighed. Samples were collected from the cardiac (heart) and *longissimus dorsi* (at the midrib area) muscles, the livers and kidney cortices and all tissues were dried at 60°C for Se analyses. The rumen content was collected, squeezed through cheesecloth and then spun out at 30000 rpm to collect the rumen bacteria, which were dried pending Se analyses. The fluorometric method of Koh & Benson (1983) was used to determine Se concentrations. A standard reference bovine liver 1577b (National Bureau of Standards, Gaithersburg, MD 20899, USA) and chicken liver (internal laboratory standard) were used to confirm the accuracy of the Se analyses.

Statistical analyses

In the designing of the investigation the same seasons in the different years were planned to be replications. However, situations varied so much between years that each trial had to be considered independently in the statistical analyses. This unfortunately decreased the degrees of freedom per treatment per trial substantially. An analysis of variance with the GLM model (Statistical Analysis System, 1994) was used to determine the significance between treatments and seasons. Initial Se concentrations in blood and plasma were included in the model as a covariance to correct for variations in initial concentrations. The level of significance was tested with Bonferroni (Samuels, 1989).

Results

At the onset of the trials the Se concentrations of the ryegrass (unfertilized) were higher than those in kikuyu (Table 1). Selenium fertilization increased the Se concentrations in both grass species above the concentrations in unfertilized kikuyu (Table 1). However, this increase was much higher ($P < 0.01$) in the ryegrass than in kikuyu ($P < 0.05$). At the first collection after fertilization, the Se concentrations in the grass were substantially higher than later during the trials. This decrease with time was more pronounced in ryegrass where the response to Se fertilization was higher than in kikuyu.

Changes in the Se concentrations of whole blood during the trials are presented in Figure 1. Selenium fertilization of kikuyu resulted in relatively small increases ($P < 0.05$) in blood Se concentrations of the lambs, reaching a concentration of *ca* 100 ng/ml, compared to the concentrations (P <

Table 1 Mean selenium concentrations of the pastures during the different seasons

			Selenium (mg/kg DM)	
Pasture	Season	n	Unfertilized	Fertilized
Kikuyu	Summer 1	8	0.034 ± 0.0027^a	0.065 ± 0.0082^b
	Summer 2	7	0.029 ± 0.008^a	0.053 ± 0.012^b
Ryegrass	Winter 1	5	0.111 ± 0.0429^c	0.485 ± 0.305^d
	Winter 2	6	0.073 ± 0.0244^c	0.325 ± 0.222^d

Values with superscripts a–d, b–d and c–d differ significantly at $P < 0.01$

0.01) of > 250 ng Se/ml in the blood of sheep on ryegrass. Blood Se concentrations in the sheep on kikuyu receiving the combined sources of Se tended to increase above those reached with one method of supplementation. No such additive increase was observed in the lambs on ryegrass (Figure 1).

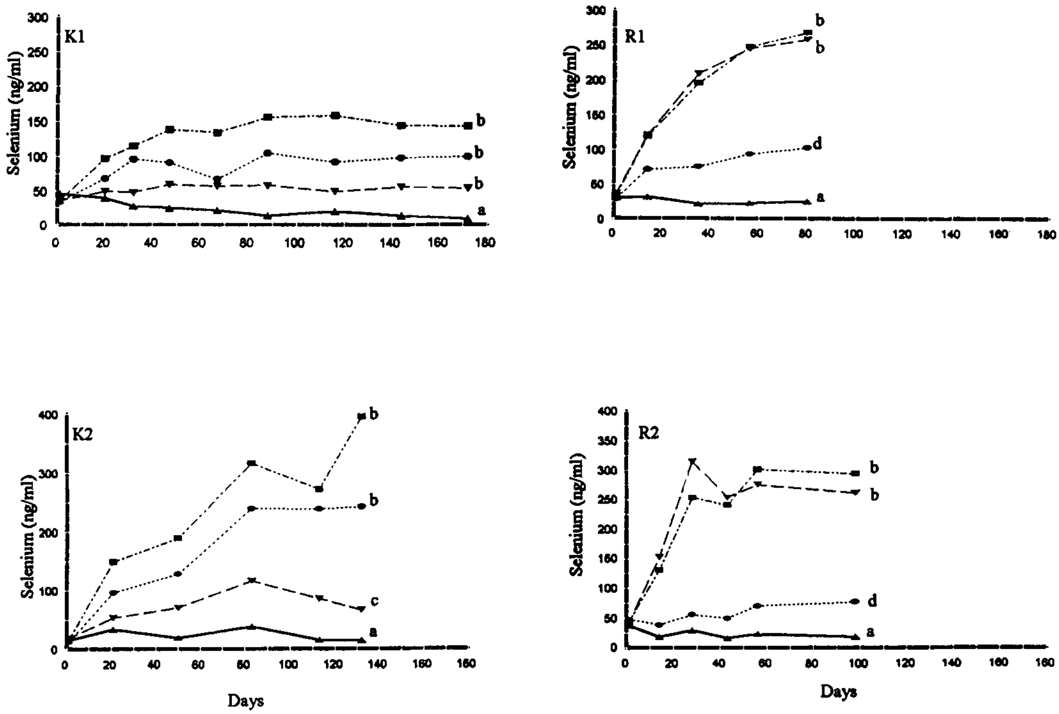

Figure 1 Blood selenium concentrations of lambs on kikuyu during summmer 1 (K1) and summer 2 (K2) and on ryegrass during winter 1 (R1) and winter 2 (R2); a–b, b–c and b–d signify significant (P < 0.05) differences. Statistical calculations based on covariance with initial blood selenium concentrations
▲5 = control; - ● = inject; -▼+ fertilize; - ■ = fertilize & inject

Within season both methods of supplementation resulted in increased (P < 0.01) Se concentrations in the liver, kidney cortex, muscle and heart above those of the respective unsupplemented controls (Tables 2–5). However, responses to Se fertilization were lower (P < 0.01) in sheep on kikuyu than on ryegrass. Responses in tissue Se concentrations to parenteral Se supplementation were relatively similar in three of the four trials. During the second kikuyu season the parenteral Se supplementation resulted in higher (P < 0.01) Se concentrations in the livers, kidneys, muscle and heart of the lambs compared to those in the parenteral treatments during the other trials. This was observed also in blood Se concentrations (Figure 1).

In the sheep on kikuyu the combined supplementation of Se resulted in non-linear increases (P < 0.01) in tissue Se concentrations above the highest in any single method of supplementation, except for the kidney cortices (Tables 2–5). On the other hand, in the sheep on ryegrass where tissue Se concentrations of the fertilized groups were much higher (P < 0.01) than those on kikuyu, the combined supplementation did not increase tissue Se concentrations above those reached from Se fertilization alone.

Table 2 Concentration of selenium (Se, ng/g DM) in livers of sheep supplemented with Se when grazing kikuyu or ryegrass pastures during different seasons

		Selenium supplementation*			
Pasture	Season	Control	Fertilize	Inject	Fert + Inject
Kikuyu	Summer 1	163^{a}	414^{b}_{p}	609^{c}_{p}	745^{d}_{q}
	Summer 2	96^{a}	393^{c}_{p}	1054^{d}_{q}	1427^{e}_{p}
Ryegrass	Winter I	124^{a}	965^{c}_{q}	438^{d}_{p}	1023^{c}_{r}
	Winter 2	137^{a}	848^{d}_{q}	403^{b}_{p}	867^{d}_{s}

* Within row (i.e. within season) values with superscripts a–b differ at $P < 0.05$ and a–c, a–d, a–e, d–c, c–e and d–e at $P < 0.01$ levels of significance

* Within column (i.e. between seasons within supplement treatment) values with subscript p–q, p–r and p–s differ at $P < 0.01$ level of significance

Table 3 Concentration of selenium (Se, ng/g DM) in kidney cortices of sheep supplemented with Se when grazing kikuyu or ryegrass pastures during different seasons

		Selenium supplementation*			
Pasture	Season	Control	Fertilize	Inject	Fert + Inject
Kikuyu	Summer 1	1783^{a}	4262^{b}_{p}	5108^{b}_{p}	5612^{b}
	Summer 2	1686^{a}	4539^{b}_{s}	6588^{C}_{q}	6478^{c}
Ryegrass	Winter 1	2682^{a}	6109^{d}_{q}	5001^{b}_{p}	6435^{e}
	Winter 2	1898^{a}	5641^{c}_{r}	4023^{b}_{p}	5434^{e}

* Within row (i.e. within season) values with superscripts b–e differ at $P < 0.05$ and a–b, a–c, a–d, a–e and b–c at $P < 0.01$ levels of significance

* Within column (i.e. between season within supplement treatment) values with subscripts p–r differ at $P < 0.05$ and p–q and q–s at $P < 0.01$ levels of significance

Table 4 Concentration of selenium (Se, ng/g DM) in skeletal muscle of sheep supplemented with Se when grazing kikuyu or ryegrass pastures during different seasons

			Selenium supplementation*		
Pasture	Season	Control	Fertilize	Inject	Fert + Inject
Kikuyu	Summer 1	67ᵃ	121ᵉ	128ᵇ	210ᶜ
			p	p	q
	Summer 2	38ᵃ	132ᵇ	202ᶜ	266ᵈ
			p	q	r
Ryegrass	Winter 1	62ᵃ	408ᵇ	144ᶜ	396ᵇ
			q	p	p
	Winter 2	72ᵃ	320ᵇ	113ᵃ	299ᵇ
			s	p	s

Within row (i.e. within season) values with superscripts a–e differ at $P < 0.05$ and a–b, a–c, a–d, b–c, b–d, c–d and e–c at $P < 0.01$ levels of significance

*Within column (i.e. between season within supplement treatment) values with subscripts q–r differ at $P < 0.05$ and p–q, p–r, p–s, r–s and q–s at $P < 0.01$ levels of significance

Table 5 Concentration of selenium (Se, ng/g DM) in cardiac muscle of sheep supplemented with Se when grazing kikuyu or ryegrass pastures during different seasons

			Selenium supplementation*		
Pasture	Season	Control	Fertilize	Inject	Fert + Inject
Kikuyu	Summer 1	97ᵃ	300ᵈ	502ᵇ	636ᶜ
			q	p	p
	Summer 2	72ᵃ	414ᵃ	972ᵇ	1054ᵇ
			q	q	r
Ryegrass	Winter 1	113ᶜ	1170ᵃ	510ᵇ	1260ᵃ
			p	p	q
	Winter 2	105ᵃ	936ᵇ	323ᵃ	907ᵇ
			s	p	s

* Within row (i.e. within season) values with superscripts a–b, a–c, c–b and c–d differ at $P < 0.01$ levels of significance

* Within column (i.e. between season within supplement treatment) values with subscripts p–s differ at $P < 0.05$ and p–q, q–s, p–r and r–s at $P < 0.01$ levels of significance

Although variations within treatments were wide, Se concentration in rumen bacteria followed the Se concentration in the diets, namely, higher concentrations ($P < 0.01$) in the Se fertilized pastures than without and again much higher in the ryegrass than in the kikuyu (Table 6). The Se concentrations of rumen bacteria in the treatments receiving the injected Se were consistently though non-significantly higher ($P > 0.05$) than in their corresponding treatments.

Table 6 Concentration of selenium (Se, ng/g DM) in rumen bacteria of sheep supplemented with Se when grazing kikuyu or ryegrass pastures during different seasons

			Selenium supplementation*		
Pasture	Season	Control	Fertilize	Inject	Fert + Inject
Kikuyu	Summer 1	51	178_{p}	56	233_{p}
	Summer 2	96^{a}	376^{b}_{s}	147	383^{b}_{s}
Ryegrass	Winter 1	186^{a}	529^{b}_{q}	226^{a}	584^{b}_{q}
	Winter 2	153^{a}	686^{b}_{r}	273^{a}	704^{b}_{r}

* Within row (i.e. within season) values with superscripts a–b differ at $P < 0.01$ level of significance

* Within column (i.e. between season within supplement treatment) values with subscripts p–q, p–r and r–s differ at $P < 0.01$ level of significance

Discussion

Selenium fertilization of ryegrass resulted in a significantly higher increase in Se concentration in the herbage than in kikuyu. This difference was reflected also in the blood, livers, kidneys, muscle and hearts of the sheep at the end of the trials. Therefore, ryegrass seems to have a much better ability than kikuyu to take up Se from the soil. Similar differences among grass species have been reported by various researchers (Davies & Watkinson, 1966; Grace & Clark, 1991; Mayland, 1994), though apparently never in kikuyu. Uptake of Se by the plant is affected by soil properties such as pH, clay content and concentration of competing ions, such as SO_4^{2-}, with Se being more available to the plant at a high than at a low soil pH (Higgins & Fey, 1993). In the present trial the ryegrass was grown on a soil with the higher pH and redox potential than the kikuyu. Therefore, the results are not completely comparable. However, since kikuyu grows better on a soil with a low pH than ryegrass, it is an accepted practice in KwaZulu-Natal to cultivate kikuyu on such acid soils (Miles, 1991). Further research is needed to confirm any species differences between kikuyu and ryegrass in ability to accumulate Se from soils. Therefore, at present the supplementation of Se via the fertilizing of kikuyu cannot be recommended at the prescribed doses, and further research is required on this as well.

Parenteral Se supplementation resulted in an approximately equivalent response in tissue Se concentrations in three of the four trials. In most lambs Deposel ($BaSeO_4$) at an application rate of 1 mg Se/kg body weight elevated Se concentrations in blood to concentrations > 60 ng/ml. This indicates a low to adequate Se status, depending on whose criterion of adequacy is used (Van Ryssen *et al.*, 1999). During the K2 season tissue and blood Se concentrations increased significantly above those of the other parenteral treatments. These lambs also lost weight especially towards the end of the trial (Van Ryssen *et al.*, 1999). A continuous release of Se from the Deposel implant could have caused this higher accumulation of Se in the tissues of these lambs that were losing weight.

In the present study blood Se concentrations in the Se supplemented ryegrass treatments increased with time to reach a plateau at between 200 and 300 ng/ml. This occurred when Se was supplemented via the fertilized grass and when both methods of supplementation were used. This non-linear accumulation of Se was observed also in the livers, heart and muscle of the ryegrass treatments. This agreed with the non-linear responses obtained by Whelan *et al.* (1994) when supplementing Se intra-ruminally and through fertilizing their pasture and by Van Ryssen *et al.* (1992) in sheep receiving a parenteral Se supplement plus dietary Se through a protein supplement. A non-linear increase in Se concentration is suggested to indicate a reduced risk to toxicity and that the body has the ability to protect itself against the accumulation of Se in reaching toxic concentrations (Whelan *et al.*, 1994). Hakkarainen (1993) furthermore stated that Se is retained in animal tissue in an inactive form. However, at higher Se intakes than those used in the present study Echevarria *et al.* (1988), Henry *et al.* (1988) and Van Ryssen *et al.* (1998) observed linear increases in tissue Se concentrations with increasing Se intake. A possible explanation is rendered by the observation by Masters *et al.* (1992) that, with increasing Se intakes, increases in Se concentrations in the blood and liver tended to reach a plateau at the intermediate Se intakes, but increased again in the liver at higher intakes.

The increased bacterial Se concentration in the sheep receiving the parenteral Se supplementation suggests that Se could have entered the rumen through the saliva. Langlands *et al.* (1986) recorded the presence of Se in the saliva, though did not record a high correlation between Se in the saliva and in blood or plasma.

It is concluded that Se supplementation via fertilizing of the pasture is effective in the case of ryegrass pastures. However, the present recommended levels of Se application does not seem to warrant its application to kikuyu pastures, and more research is required to clarify the situation with kikuyu.

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