

## Supplementation of selenium to sheep grazing kikuyu or ryegrass: I. Selenium status of unsupplemented sheep and animal performance upon supplementation

J.B.J. van Ryssen,\* R.J. Coertze

Department of Animal & Wildlife Sciences, University of Pretoria, Pretoria, 0002 South Africa

J.F. de Villiers

Cedara Agricultural Research Station, KwaZulu-Natal Department of Agriculture,  
Private Bag X9059, Pietermaritzburg, 3200 South Africa

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The selenium (Se) status of lambs grazing kikuyu (*Pennisetum clandestinum*) or ryegrass (*Lolium multiflorum*) in the KwaZulu-Natal Midlands was determined during four trials and their growth response to Se supplementation was measured. Two methods of Se supplementation were evaluated using a 2 × 2 factorial experimental design per season: a control, Se supplemented via fertilizing the pasture and/or injecting the lambs with a long-acting parenteral Se product. The Se concentrations in the whole blood of the unsupplemented groups at the end of the trial varied between 9 and 26 ng/ml, those in plasma between 5 and 15 ng/ml and in the livers between 96 and 163 ng/g DM. From these parameters it was concluded that the lambs were in state of Se deficiency, even though no symptoms of Se deficiency were observed. The lambs on the kikuyu did not grow well and even lost weight during the second summer. Despite the low weight gain during the first summer, a better ( $P < 0.05$ ) growth response was obtained in the group receiving the parenteral Se supplementation above that of the unsupplemented group and the one grazing the Se fertilized kikuyu. The cumulative weight gains in both the Se fertilized groups during one ryegrass trial were higher ( $P < 0.05$ ) than those in the control and parenteral supplemented groups. It was concluded that, although Se supplementation improved the growth of the lambs, the response varied between years. It was suggested that the poor growth of the lambs in some years was caused by factors other than a selenium deficiency and had to be overcome to obtain a positive response to Se supplementation.

**Keywords:** Selenium, sheep, kikuyu, ryegrass, KwaZulu-Natal Midlands, parenteral, fertilized

\* To whom correspondence should be addressed.

### Introduction

Conditions conducive to a selenium (Se) deficiency in grazing livestock are well-drained acid soils in high rainfall areas and improved pasture species which are regularly fertilized (Langlands *et al.*, 1991). Acid soil conditions are widespread in the high rainfall areas of southern Africa, including the KwaZulu-Natal Midlands (Miles, 1991). In a survey on the Se concentration of ryegrass and veld grass in KwaZulu-Natal, Higgins & Fey (1993) concluded that a Se deficiency ( $< 0.1$  mg Se/kg dry matter, DM) is likely in animals grazing these grasses. However, clinical signs of a Se

deficiency in sheep on cultivated pastures have not been reported in this region. The more likely situation would be marginal or subclinical deficiencies, the consequences of which may be underestimated because clinical signs were absent (Langlands *et al.*, 1991; Whelan *et al.*, 1994a). A positive response in performance of Se supplementation is the most reliable way of detecting the existence of a marginal Se deficiency in animals in a region.

The objective of this investigation was to obtain more scientific information on the Se status of sheep in the KwaZulu-Natal Midlands and to evaluate the effectiveness of different methods of supplementing Se. The Se status of unsupplemented lambs and growth responses to supplementation are reported in this article.

## Materials and methods

### Animals and treatments

Four separate grazing trials were conducted with SA Mutton Merino weaner lambs. During two summer periods (K1 and K2) lambs grazed dryland kikuyu (*Pennisetum clandestinum*) at a stocking rate of 35 lambs/ha and during two winter periods (R1 and R2) the lambs grazed irrigated annual Italian ryegrass (*Lolium multiflorum* cv. Midmar) at a stocking rate of 50 lambs/ha. In each trial 20 lambs (24 lambs were used during the second summer) were divided randomly into four treatment groups according to a 2 × 2 factorial experimental design: an unsupplemented control; 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); one subcutaneous injection of a slow-releasing Se treatment (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 Se injection plus the Se fertilized pasture. Each group of lambs was slaughtered at the end of a 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, altitude 1067 m, average annual rainfall 885 ± 142 mm. The kikuyu was grown on a Hutton/Devon soil and the ryegrass in a vleiland on a Katspruit soil. Details of the pasture treatments are presented elsewhere (Van Ryssen *et al.*, 1999).

The sheep were weighed weekly. Water and feed were not withheld prior to the weighing. The lambs were vaccinated according to a local management program. All lambs were dosed a wide-spectrum anthelmintic when required, based on roundworm egg counts in pooled faecal samples. This was done at the Allerton Veterinary laboratory.

### Sampling and chemical analyses

At approximately monthly intervals during each trial, blood samples were collected by jugular venipuncture in evacuated tubes containing lithium heparin as anticoagulant. At slaughter, tissues were collected and dried at 60°C. Details are reported in Van Ryssen *et al.* (1999). The whole blood, plasma, liver and grass samples were assayed for Se using the fluorometric method of Koh & Benson (1983). Packed cell volume (PCV) was measured at each collection, using a microhaematocrit. Blood was collected once at the end of each trial to determine the calcium (Ca), inorganic phosphorus (P), magnesium (Mg), sodium (Na), copper (Cu) and zinc (Zn) concentrations in plasma on an atomic absorption spectrophotometer, except for inorganic P where the AOAC (1985) method was used. Plasma aspartate transaminase (AST; EC 2.6.1.1) and creatine kinase (CK; EC 2.7.3.2) activities were measured using the respective Boehringer Mannheim analytical kits (Boehringer Mannheim GmbH Diagnostica, Germany).

## Statistical analyses

The lambs on the kikuyu were approximately six months older than those on ryegrass. A direct comparison in growth performance between pasture types was therefore not attempted. In designing 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 pasture substantially. Two sheep were stolen during the second ryegrass trial, one from the control and one from the Deposel treatment. An analysis of variance with the GLM model (Statistical Analysis System, 1994) was used to determine the significance between treatments and seasons. Initial body weights were included in the model as a covariance to correct for variations in initial weights. Least square means were calculated. The level of significance was tested with Bonferroni (Samuels, 1989).

## Results

The average Se concentration in unfertilized kikuyu in Summer 1 was  $0.034 \pm 0.0027$  mg Se/kg DM and in Summer 2,  $0.029$  mg/kg DM. In Winter 1 the unfertilized ryegrass contained  $0.111 \pm 0.0429$  mg Se/kg DM and  $0.073 \pm 0.0244$  mg Se/kg DM in Winter 2. The Se concentrations in whole blood and plasma at the onset and in the unsupplemented treatments at the end of each trial are presented in Table 1. The unsupplemented lambs in all four trials experienced a gradual decrease in Se concentration with time. The liver Se concentrations in the unsupplemented groups are presented also in Table 1.

**Table 1** Selenium (Se) status of all lambs at the onset and of the unsupplemented lambs at the end of each trial

|          | Onset of trials* |                 | End of trials (unsupplemented groups)** |                 |                  |
|----------|------------------|-----------------|---|-----------------|------------------|
|          | Blood Se ng/ml   | Plasma Se ng/ml | Blood Se ng/ml                          | Plasma Se ng/ml | Liver Se ng/g DM |
| Kikuyu 1 | 37.9 ± 14.2      | 24.2 ± 9.95     | 9.0 ± 15.95                             | 8.2 ± 5.59      | 163 ± 56.8       |
| Kikuyu 2 | 16.4 ± 7.73      | 19.2 ± 14.61    | 14.9 ± 10.14                            | 5.2 ± 6.38      | 96 ± 51.8        |
| Rye 1    | 32.2 ± 11.82     | 28.3 ± 15.85    | 26.2 ± 12.13                            | 9.8 ± 6.32      | 124 ± 63.5       |
| Rye 2    | 42.1 ± 21.6      | 20.1 ± 6.45     | 19.0 ± 11.5                             | 15.3 ± 9.26     | 137 ± 63.5       |

\*n = 20, except for kikuyu 2 where n = 24

\*\*n = 5, except for kikuyu 2 where n = 6

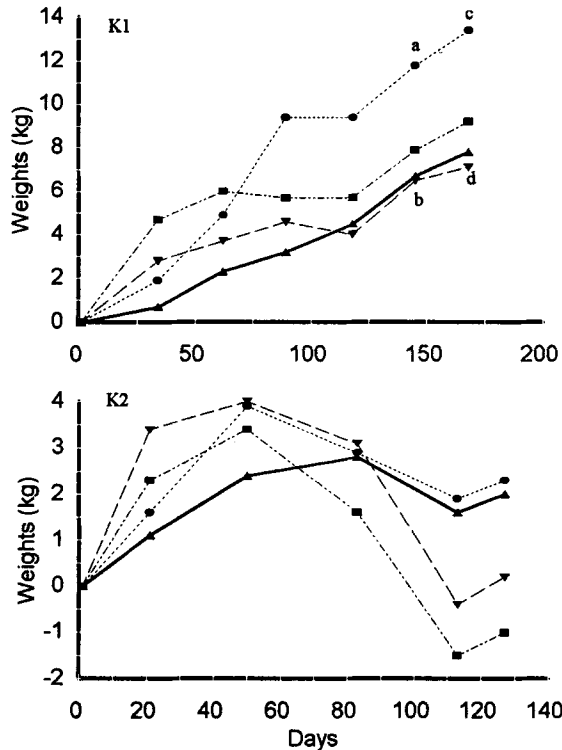
Within pasture type the average daily gains (ADG) of the lambs did not differ significantly among treatments (Table 2). During the second season on kikuyu the lambs lost weight towards the end of the trial and in total gained very little in mass. The cumulative weights of the lambs during the different seasons are depicted in Figures 1 and 2. At the last two weighings during the K1 season the lambs receiving the parenteral Se supplementation gained more ( $P < 0.05$ ) than the lambs in the control and the Se fertilized treatments. At the last two weighings during the R2 season the lambs

**Table 2** Average daily gains in weight of lambs during the different seasons

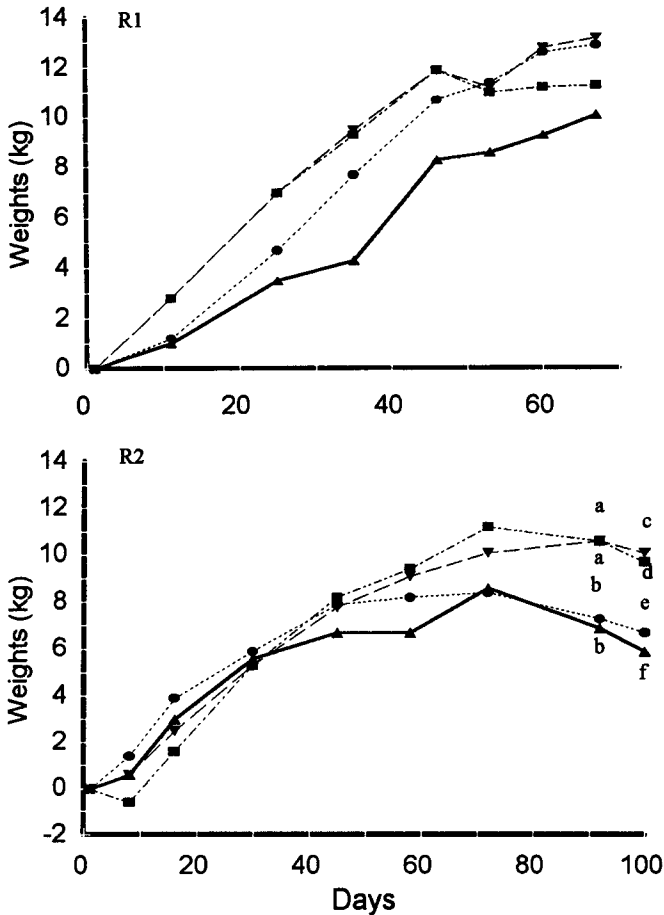
|          | Average daily gain* (g) |           |        |               | SEM  |
|----------|-------------------------|-----------|--------|---------------|------|
|          | Control                 | Fertilize | Inject | Fert + Inject |      |
| Kikuyu 1 | 48                      | 42        | 79     | 51            | 10.5 |
| Kikuyu 2 | 20                      | 3         | 17     | -5            | 7.6  |
| Rye 1    | 211                     | 209       | 205    | 179           | 22.2 |
| Rye 2    | 60                      | 102       | 90     | 98            | 10.0 |

\* Within season differences were not significantly different

on the Se fertilized and fertilized plus parenteral Se treatments gained more ( $P < 0.05$ ) than the lambs in the other two treatments. Within season the fresh carcass, liver and kidney masses did not differ significantly among treatments.



**Figure 1** Cumulative weight gains of lambs on kikuyu (K1 = summer 1; K2 = summer 2) -▲= control; -● = inject; -▼+ fertilize; -■ = fertilize & inject, a-b and c-d signify significant ( $P < 0.05$ ) differences. Statistical calculation based on covariance with initial weight



**Figure 2** Cumulative weight gains of lambs on ryegrass (R1 = winter 1; R2 = winter 2) -▲= control; -●= inject; -▼+ fertilize; -■= fertilize & inject, a-b, c-e, c-f, d-f signify significant ( $P < 0.05$ ) differences. Statistical calculation based on covariance with initial weight

At the end of the trials the average mineral concentrations in plasma for both kikuyu trials were:  $93.4 \pm 7.09$  mg Ca;  $3.4 \pm 0.53$  g Na;  $20.7 \pm 2.58$  mg Mg;  $0.96 \pm 0.229$  mg Cu and  $1.03 \pm 0.311$  mg Zn/litre plasma; and for the ryegrass trials:  $103.8 \pm 9.8$  mg Ca;  $66 \pm 11.8$  mg P;  $19.9 \pm 2.46$  mg Mg;  $1.05 \pm 0.366$  mg Cu and  $0.80 \pm 0.138$  mg Zn/litre plasma. Enzyme activities in plasma at the end of the trials were: kikuyu trials: AST  $50.1 \pm 9.09$  U/litre and CK  $39.5 \pm 19.69$  U/litre and ryegrass trials: AST,  $92.5 \pm 25.84$  U/litre; CK,  $119.6 \pm 22.2$  U/litre. The PCV of the sheep on kikuyu at the end of the two seasons was  $30.4 \pm 1.71\%$  and those on ryegrass  $35.1 \pm 1.83\%$ . None of these parameters differed statistically between treatments within season.

## Discussion

Dietary Se requirements of sheep are generally quoted to be between 0.1 and 0.3 mg/kg DM (Mayland, 1994; Puls, 1994). However, under grazing conditions in New Zealand no response to Se supplementation of sheep is expected at dietary concentrations of  $> 0.03$  mg Se/kg DM (Grace &

Clark, 1991). This pertains to the New Zealand situation with specific dietary antagonists and synergists of Se and specific challenges to Se functions in the body. The average Se concentration of the unfertilized kikuyu during the two summer seasons of our study was  $< 0.03$  mg/kg DM, indicating a potential Se deficiency in the animals. The average Se concentrations in the ryegrass during these two winter seasons were 0.073 and 0.111 mg/kg DM. These were substantially higher than the Se concentrations measured by Higgins & Fey (1993) in ryegrass in KwaZulu-Natal. However, in general, pasture samples are considered unreliable as the sole basis for predicting the Se status of grazing animals (Whelan *et al.*, 1994a).

At the onset of the present trials, Se concentrations ranged from 16 to 42 ng/ml in blood and 19 to 28 ng/ml in plasma. During all four trials these concentrations in the treatments receiving no supplemental Se (the controls) decreased with time to an average of 11.8 ng/ml in blood and 6.7 ng/ml in plasma of the sheep grazing kikuyu, and 22.6 ng/ml in blood and 12.5 ng/ml in plasma of the sheep grazing ryegrass. From the literature there appears to be no consensus on what concentrations of Se in ovine blood and plasma indicate a Se deficiency (Whelan *et al.*, 1994a). For New Zealand conditions, 10.3 ng/ml blood is considered deficient and 19.7 ng/ml blood as adequate (Grace & Clark, 1991). In New South Wales, Australia,  $< 20$  ng Se/ml blood is considered as indicative of a Se deficiency (Langlands *et al.*, 1991; Donald *et al.*, 1993) and in Western Australia no growth response to supplementation is expected at blood Se concentrations of  $> 30$  ng/ml (Peter *et al.*, 1980). Whelan *et al.* (1994b) recommended for Western Australia that values of  $> 60$  ng/ml in whole blood and  $> 40$  ng/ml in plasma be used as criteria of adequacy. Based on Canadian data, Puls (1994) considered Se concentrations of  $< 50$  ng/ml blood and  $< 30$  ng/ml serum as deficient and  $> 120$  ng/ml blood and  $> 80$  ng/ml serum as adequate. Maas & Koller (1985), in the United States of America, tabulated whole blood Se concentrations of 10 to 40 ng/ml as indicative of a deficient status where animals will always benefit from Se supplementation and 50 to 60 ng/ml as marginal where Se supplementation is often beneficial.

From the literature hepatic Se concentrations indicating a marginal deficiency in sheep varied substantially, for instance: 200–500 ng/g DM (Caple & McDonald, 1983); 158–316 ng/g DM (Archer & Judson, 1994) and 500–833 ng/g DM (assuming fresh liver contains 30% DM) (Puls, 1994). At slaughter the liver Se concentrations in the respective control treatments in the present study were well below 200 ng/g DM, indicating an acute Se deficiency. This confirms the results in the blood analyses at least for the lambs on kikuyu. Furthermore, Se supplementation elevated Se concentrations in blood and liver to concentrations which could be considered as adequate in all treatments, except in the kikuyu topdressed with Se (Van Ryssen *et al.*, 1999).

Symptoms of an acute Se deficiency were not observed, nor did plasma enzyme activities reveal any tissue damage which could be attributed to a Se deficiency (Pherson, 1993). The plasma CK activities were higher than the norm (Puls, 1994), though did not show any treatment effect. Factors provoking clinical symptoms such as a high concentration of polyunsaturated fatty acids in the diet, unaccustomed exercise, rapid growth in young animals and a challenge to the immune system (Pherson, 1993) might not have been present in our investigation.

Selenium supplementation resulted in a significant increase in Se concentrations of the animal tissues in most of the treatments (Van Ryssen *et al.*, 1999). However, supplementation resulted in a significant improvement in weight gain in only some of the supplemented treatments, including when the ryegrass which contained 0.073 mg Se/kg DM was fertilized with Se. These growth responses supported the observation by Van Ryssen *et al.* (1992) that lambs grazing cultivated pastures in the Midlands of KwaZulu-Natal should benefit from Se supplementation. Possible reasons for the poor response in the present investigation may be suggested. However, it must be accepted

that the low number of animals per treatment per season would restrict the potential to demonstrate responses to treatments.

Compared to the growth rate of lambs at the same location (De Villiers *et al.*, 1994), in the present investigation a low growth rate was recorded for the lambs on kikuyu, with even a loss in weight towards the end of the one grazing season. Considering the low Se concentrations in the blood and liver, positive responses to Se supplementation could have been expected in both seasons. It seems as if some other factors restricting the growth of the lambs on kikuyu during the one season, overshadowed a possible response to Se supplementation (Peter, 1980). The plasma mineral concentrations of the lambs did not indicate a deficiency in any of the other minerals tested. Based on the faecal egg counts the lambs could not have suffered from internal parasite infestation, though the PCV concentrations of the lambs on kikuyu were lower than those of the sheep on ryegrass. Hunter *et al.* (1982) pointed out that responses to supplementation of deficient situations could be masked by low energy intakes during critical periods. In the lambs on ryegrass a growth response to Se supplementation was recorded in the year when the average daily gains were low, compared to the other season. However, during both seasons the Se concentrations in the blood and liver of the control groups indicated a Se deficiency. The reason for lack of response during the first season is unclear, though, according to Langlands *et al.* (1991) and Whelan *et al.* (1994a) marginal Se deficiencies may not occur every year or in all situations.

From this and previous studies (Van Ryssen *et al.*, 1992; Higgins & Fey, 1993) it can be accepted that a Se deficiency is likely to exist in the KwaZulu-Natal Midlands in animals grazing cultivated pastures. However, Van Saun (1990) emphasized that the existence of a Se deficiency should be well established prior to initiating a supplementary program, even where an area has been identified as potentially deficient. Since the reliability of biochemical indicators is situation specific, it seems advisable to establish guidelines for the KwaZulu-Natal Midlands of blood and plasma Se concentrations indicative of a Se deficiency in the region.

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