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A long-acting injectable Se/Vitamin B_{12} product to prevent Se and Co deficiency in lambs

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ABSTRACT

The efficacy of a novel injectable formulation containing barium selenate ($BaSeO_4$) and microencapsulated Vitamin B_{12} was evaluated in groups of lambs (n=10) over 246 days in terms of changes in blood Se and serum Vitamin B_{12} concentrations as well as growth responses. Mean blood Se and serum Vitamin B_{12} concentrations of the untreated control lambs were 125 ± 9 nmol/L and 508 ± 21 pmol/L, respectively. The formulation increased blood Se to 2100 nmol/L at day 64 before decreasing to 761 nmol/L at day 246. Likewise, serum Vitamin B_{12} reached a peak of 1950 pmol/L at day 31 before decreasing to 577 pmol/L at day 246. The treatment significantly increased ($P < 0.05$) daily weight gains from 125 ± 4 to 136 ± 4 g/day. An injectable Se/Vitamin B_{12} product containing 12 mg Se as $BaSeO_4$ and 3 mg Vitamin B_{12} per dose administered at docking was effective in increasing and maintaining the Se and Vitamin B_{12} status of lambs for at least 246 days.

Keywords: lambs; blood; serum; growth response; selenium; Vitamin B_{12}.

INTRODUCTION

Selenium (Se) and cobalt (Co) deficiencies cause poor growth rates and mortality in lambs (Andrews, 1954; Metherell *et al.*, 1996; Grace & Knowles, 2002). Cobalt is required by rumen micro-organisms for the synthesis of Vitamin B_{12}, of which 3-10% of the vitamin is absorbed from the small intestine and stored in liver. Vitamin B_{12} is a cofactor for two enzymes, namely methylmalonyl coenzyme A mutase and methionine synthase, which are associated with the energy and protein metabolism of micro-organisms and lambs (Rice *et al.*, 1989; Kennedy *et al*., 1992). Clinical signs of Co deficiency are loss of appetite, poor growth and a watery eye discharge. Pastures containing <0.08 mg Co/kg DM are Co deficient and the serum and liver Vitamin B_{12} concentrations of lambs grazing these pastures can be <250 pmol/L and <110 μmol/kg fresh tissue respectively (Andrews, 1954; Grace & Sinclair, 1999).

Selenium is a cofactor for the enzyme glutathione peroxidase which, through its antioxidant properties, maintains the integrity and function of cell membranes as well as important compounds such as lipids, nucleic acids and proteins (Tinggi, 2003). Clinical signs of Se deficiency include white muscle disease. Pastures containing <0.03 mg Se/kg DM are Se deficient, and as a result the grazing lambs can have blood and liver Se concentrations of <130 nmol/L and <250 nmol/kg fresh tissue, respectively (Grant & Shepherd, 1983).

Using controlled-release technology, a novel dual Se/Vitamin B_{12} injectable animal remedy has been developed with advantages over conventional therapies in terms of cost effectiveness and reduced labour. The product complements a long-acting injectable Vitamin B_{12} previously developed to prevent Co deficiency in lambs (Grace & Lewis, 1999). This report describes the efficacy of a new Se/Vitamin B_{12} injectable animal remedy in terms of changes in blood Se, serum Vitamin B_{12} and growth responses in lambs.

MATERIALS AND METHODS

Animals

The study was carried out in 2000/2001 on the AgResearch Flock House Research Farm, near Bulls, in the southern part of the North Island of New Zealand. On the basis of their blood Se concentrations 50 mixed-sex Romney x Suffolk lambs were eartagged and randomly allocated to two groups of 25 lambs (mean age 4 weeks and live weight 13.9 kg) at docking in early October 2000.

Treatments

The lambs were injected subcutaneously in the anterior neck region (Day 1) and the two treatments were.

Group 1. 1 ml of peanut-oil-based vehicle with no Se or Vitamin B_{12} (control).

Group 2. 1 ml of product containing 12 mg Se as barium selenate and 3 mg Vitamin B_{12} in a peanut-oil-based vehicle.

All lambs with their dams were grazed and managed as a single group until weaning (January 2001), after which the lambs continued to be grazed as a single group for a further six months. All lambs were weighed at about monthly intervals. The study was approved by the Crown Research Animal Ethics Committee (Palmerston North, New Zealand).

Collection of samples

At day -10, all lambs were bled from the jugular vein using a vacutainer containing EDTA to provide initial blood Se data. Just prior to treatment, and on days 31, 64, 100, 128, 158, 191, 219 and 246, monitor lambs from each group (n=10) were bled using plain and EDTA vacutainers. Serum was separated by centrifuging at 2000 *g* for 20 minutes. The whole blood and serum were then stored at 4°C before being analysed within 7 days. Herbage was cut monthly from 100-150 m transects across the paddocks grazed by the sheep. The unwashed bulk herbage was mixed thoroughly and subsampled before being dried at 60°C for 48 hours, ground and stored in air-tight plastic containers.

Analytical methods

Vitamin B_{12} concentrations in serum were determined by the Becton Dickinson radioassay method (Millar & Albyt, 1984) and blood Se by a semi-automated fluorometric method (Watkinson, 1979). Pasture Co and Se were determined by graphite furnace atomic absorption spectroscopy (Jago *et al.*, 1971) and a semi-automated fluorometric method (Watkinson, 1979), respectively.

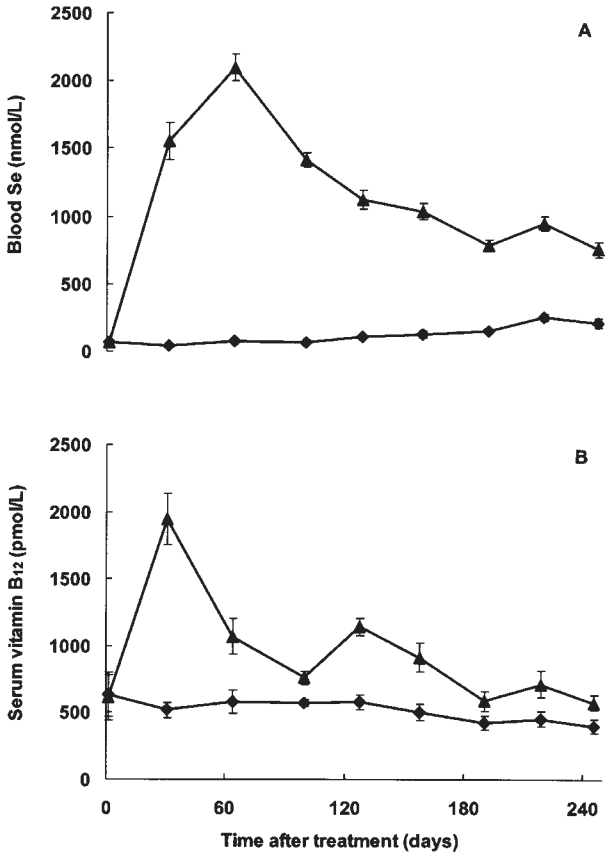
Statistical analysis

Treatment differences in blood Se and serum Vitamin B_{12} concentrations were determined at each sampling period by ANOVA using the statistical procedures of SAS 6.12 (SAS Institute Inc., Cary NC, USA).

RESULTS

The mean (range) pasture Se concentration was 0.02 (0.01-0.04) mg/kg DM while the mean (range) pasture Co concentration was 0.07 (0.05 – 0.12) mg/kg DM. Initial mean blood Se concentration of the untreated control lambs was 67 ± 6.1 nmol/L but this decreased to 40 ± 2.9 nmol/L at day 31 before slowly increasing to 213 ± 33 nmol/L at day 246. The effect of the Se/Vitamin B_{12} injection on blood Se concentrations is shown in Figure 1a. Treatment with $BaSeO_4$ significantly increased blood Se concentrations to 2100 ± 100 nmol/L at day 64 before they decreased to 761 ± 58 nmol/L at day 246. This end value was 3.5 times greater ($P < 0.01$) than that of the untreated lambs.

FIGURE 1. Effect of an injectable Se/Vitamin B_{12} given at docking on (A) blood Se and (B) serum Vitamin B_{12} concentrations (± SEM) of control (◆) and treated (▲) lambs.



The initial serum Vitamin B_{12} concentration among control lambs was 635 ± 164 pmol/L but this decreased to 397 ± 50 pmol/L at day 246. The effect of the Se/Vitamin injection on serum Vitamin B_{12} concentrations is shown in Figure 1b. The serum Vitamin B_{12} concentrations increased to 1950 ± 188 pmol/L at day 31 before decreasing to 577 ± 56 pmol/L at day 246. This was 1.6 times greater ($P < 0.01$) than that of the untreated lambs. The average daily weight gain of the Se/Vitamin B_{12} treated lambs was 136 ± 4 g/day, a rate significantly greater ($P < 0.05$) than the 125 ± 4 g/day for the untreated lambs.

DISCUSSION

A long-acting dual Se/Vitamin B_{12} product, administered at docking to lambs aged 3 to 5 weeks, is a novel and effective approach to preventing Se and Co deficiency in lambs. The product described in this study was selected as the best formulation after evaluating a number of Se chemical forms and depot sizes.

The pastures grazed by the ewes and lambs in this study were Se deficient (i.e., <0.03 mg Se/kg DM) and the mean initial blood Se concentration of all lambs was <70 nmol/L. Mean blood Se concentrations of untreated animals remained <150 nmol/L for most of the study. A liveweight response of 11 g/day to the Se/Vitamin B_{12} supplement was observed. Other Se supplementation growth response trials with lambs in New Zealand have also shown that lambs with blood Se concentrations <130 nmol/L are deficient and will likely respond to Se supplementation with economically valuable growth increases of >10 g/day (Grace & Knowles, 2002).

The mean serum Vitamin B_{12} concentration of the untreated lambs during the study was adequate (>450 pmol/L) and, therefore, no growth response to Vitamin B_{12} supplementation would be expected. In earlier studies where the initial mean serum Vitamin B_{12} concentration was <179 pmol/L, a growth response in lambs was observed to a long-acting injectable Vitamin B_{12} similar to this product (Grace *et al.*, 2003).

The changes in blood Se and serum Vitamin B_{12} concentrations show that a Se/Vitamin B_{12} injection providing about 1 mg Se/kg LW and 0.2 mg Vitamin B_{12}/kg LW at docking was effective at increasing and maintaining the Se and Vitamin B_{12} status of lambs over 246 days. The product has a large safety margin because blood Se concentrations must exceed 13,000 nmol/L to be within the toxic range (Grace, 1994) and Vitamin B_{12} is considered non-toxic (Anon., 1963).

Many factors can influence the release rate of bioactives from a controlled release system. The release rates in this dual product, as measured by changes in blood Se and serum Vitamin B_{12} concentrations, are similar to those of the single Se and Vitamin B_{12} products when injected separately into sheep (Ankenbauer-Perkins *et al.*, 2000; Grace & West, 2000).

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