An Evaluation of Long Acting Injectable Microencapsulated Vitamin B\textsubscript{12} to Prevent Cobalt Deficiency in Grazing Ruminants

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\textbf{ABSTRACT:} The efficacy of a long acting Vitamin B\textsubscript{12} consisting of microencapsulated Vitamin B\textsubscript{12} in a polymer of organic acids, when injected subcutaneously or intramuscularly into the anterior neck region, was evaluated in lambs and calves. The serum and liver Vitamin B\textsubscript{12} concentrations of the treated lambs (0.12 to 0.25 mg Vitamin B\textsubscript{12} /kg LW) were increased and maintained, being at least 40% higher than the untreated animals at days 210 or 249. In Co deficient lambs an injection of microencapsulated Vitamin B\textsubscript{12} increased tissue Vitamin B\textsubscript{12} concentrations as well as their growth rates (75 v 175 g/day). Likewise the serum and liver Vitamin B\textsubscript{12} concentrations of treated calves (0.12 to 0.30 mg Vitamin B\textsubscript{12} /kg LW) were increased and maintained, when compared to untreated animals for at least 110 and 150 days respectively.

\textbf{Key Words:} Lambs, Calves, Injection, Vitamin B\textsubscript{12}, Serum, Liver, Growth rate

\textbf{INTRODUCTION}

Cobalt deficiency is an important problem, particularly in lambs, for grazing livestock in New Zealand and Australia (Lee, 1991; Grace, 1994). Low Co intakes reduce the synthesis of Vitamin B\textsubscript{12} in the reticulorumen, which results in low tissue Vitamin B\textsubscript{12} concentrations, and impaired energy and protein metabolism (Somers and Gawthorne, 1969). The deficiency is characterised by the loss of appetite, reduced liveweight and wool production (Andrews et al., 1966), and increased neonatal mortality in lambs born to Co-deficient ewes (Fisher and MacPerson, 1991). An impairment of immunity in lambs and calves has also been demonstrated (Ferguson et al., 1989). Other conditions associated with low Vitamin B\textsubscript{12} status are polioencephalomalacia, ovine white liver disease, phalaris staggers in sheep, and infertility and low milk production in cattle (Grace, 1994).

Strategies to prevent Co deficiency include topdressing pastures annually with 350g cobalt sulphate/ha but on some soils with a high Mn content the Mn reduces the plant Co uptake. Injections into sheep with water soluble Vitamin B\textsubscript{12} are effective for about 4 weeks, while drenches with Co solutions are effective for less than 2 weeks (Grace, 1998). Cobalt pellet administration with a grinder in sheep or cattle is effective for at least a year (Judson et al., 1995). Recently a long acting injectable Vitamin B\textsubscript{12} preparation consisting of Vitamin B\textsubscript{12} microencapsulated in a polymer of organic acids has been developed (Grace and Lewis, 1999).

This paper presents data on the efficacy of an injectable microencapsulated Vitamin B\textsubscript{12}.Changes in serum and liver Vitamin B\textsubscript{12} concentrations in lambs and calves and growth responses in lambs are reported.

\textbf{MATERIALS AND METHODS}

\textbf{Animals}

For trial 1 (1997/98) and trial 2 (1998/99) 3 groups of 10 animals were randomly selected from 200 weaned lambs aged 18-20 weeks, ear-tagged and weighed. The microencapsulated Vitamin B\textsubscript{12} was then administered as an injection into the anterior neck region. Treatments for trial 1 were group one, untreated control given vehicle material without Vitamin B\textsubscript{12}; group two, 6 mg Vitamin B\textsubscript{12} subcutaneously; and group three, 6 mg Vitamin B\textsubscript{12} intramuscularly. Treatments for trial 2 were group one, untreated control; groups two, 3 mg Vitamin B\textsubscript{12} subcutaneously; and group three, 6 mg Vitamin B\textsubscript{12} subcutaneously. After treatment all lambs were grazed as a single group for at least 244 days on a ryegrass/white clover pasture at the AgResearch farm near Palmerston North.

For trial 3, (1998/99) 2 groups of 30 lambs were randomly selected from 60 four to five-week old lambs, ear-tagged and weighed in December 1998. The treatments were group one, untreated control; and group two, 6 mg Vitamin B\textsubscript{12} subcutaneously. After treatment the lambs and their dams were grazed together until weaning in January 1999 after which they continued to be grazed as a single group, on ryegrass/white clover pasture, on a property near Gore (Invercargill), until they were slaughtered at day 146.

For trial 4, 50 calves were ear-tagged, weighed and randomly placed into 5 groups of 10 in July 1998. Treatments were group one, untreated control; group two, 12 mg Vitamin B\textsubscript{12}; group three, 18 mg Vitamin B\textsubscript{12}; group four, 24 mg Vitamin B\textsubscript{12} and group five, 30 mg Vitamin B\textsubscript{12}. After treatment all calves were grazed as a single group on ryegrass/white clover pasture on the AgResearch farm near Bulls (Palmerston North). All lambs and calves were weighed at about monthly intervals.
Collection of Samples
Pasture samples were collected at about two-monthly intervals for Co determinations. In trial 1 the blood samples were collected using a 10 ml vacutainer from the jugular vein just prior to treatment and on days 27, 47, 68, 83, 115, 142, 180 and 210, while for trial 2 the blood samples were collected just prior to treatment and on days 16, 36, 63, 90, 120, 166, 203 and 249. Trial 3 blood samples were collected, just prior to treatment and on days 95 and 146. Livers were collected in trials 1 and 3 when the weaned and four to five-week old lambs were slaughtered at days 244 and 146 respectively. In the calf trial, the blood and liver biopsies were collected just prior to and on days 44, 110, 188 and 244.

Analytical methods
The Vitamin B\textsubscript{12} concentrations in the serum and liver were determined using the Becton Dickinson radio-assay method (Millar and Albyt, 1984) and pasture Co by graphite furnace atomic absorption spectroscopy (Jago et al., 1971).

Statistics
Differences between treatments were determined by analysis of variance of repeated measures over time using the Statistical Analysis System (SAS, 1987).

RESULTS
Animal Liveweight
The growth of the weaned lambs in trials 1 and 2 was not improved by the Vitamin B\textsubscript{12} supplementation. The initial liveweights and growth rates of the weaned lambs were 28.4 ± 1.5 and 29.7 ± 1.7 kg and 100 ± 5.8 and 124 ± 6.5 g/day respectively. A highly significant (P<0.001) growth response was observed in four to five-week old lambs (n=30) in trial 3 where the initial liveweight was 12.5 ± 0.42 kg while the growth rates for the untreated and Vitamin B\textsubscript{12} supplemented lambs were 75.0 ± 60.4 and 175 ± 4.9 g/day respectively. No growth response to Vitamin B\textsubscript{12} supplementation occurred in the calves (trial 4) where the initial liveweight was 110 ± 3.5 kg and the growth rate was 0.77 ± 0.08 kg/day.

Pasture Co
The mean Co concentrations of the pastures grazed by the weaned, four to five-week old lambs and calves were 0.11, 0.04 and 0.16 mg Co/kg DM respectively.

Tissue Vitamin B\textsubscript{12}
The observed changes in the serum and liver Vitamin B\textsubscript{12} concentrations in lambs and calves are presented in Tables 1 and 2. The 6 mg injection in the weaned lambs significantly increased the serum Vitamin B\textsubscript{12} concentration, which reach a peak at days 36-47 before slowly decreasing to be at least 43% greater than the observed concentration on the untreated lambs at days 210 or 249. Liver Vitamin B\textsubscript{12} concentration was also significantly increased. Subcutaneous and intramuscular injections were equally effective. Serum Vitamin B\textsubscript{12} response in the 3 mg Vitamin B\textsubscript{12} lambs was less than in the 6 mg Vitamin B\textsubscript{12} animals but significantly greater than in the controls. The 6 mg Vitamin B\textsubscript{12} injection increased serum and liver Vitamin B\textsubscript{12} concentration by 63% and 273% respectively in Co-deficient four to five-week old lambs at day 146.

In calves the increased amounts of injected Vitamin B\textsubscript{12} progressively increased the serum Vitamin B\textsubscript{12} concentration which peaked at day 44 and then decreased so that by day 188 animals on treatment showed a similar concentration. A similar pattern was observed for the liver Vitamin B\textsubscript{12} concentration except that it reached its highest concentration at day 110 or 188. There were no significant treatment differences by day 188.
Table 1. Effect of a long acting injectable Vitamin B<sub>12</sub> on serum (pmol/L) and liver Vitamin B<sub>12</sub> (nmol/kg FW) concentrations in lambs (n=10)

<table>
<thead>
<tr>
<th>Day</th>
<th>1</th>
<th>16-27</th>
<th>36-47</th>
<th>62-68</th>
<th>83-95</th>
<th>115-120</th>
<th>142-166</th>
<th>180</th>
<th>203-210</th>
<th>249</th>
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<tr>
<td></td>
<td></td>
<td>Serum Vitamin B&lt;sub&gt;12&lt;/sub&gt;</td>
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<td>Liver Vitamin B&lt;sub&gt;12&lt;/sub&gt; (day 244)</td>
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<td>Trial 1 (1997/98) weaned lambs</td>
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<td>Control</td>
<td>790</td>
<td>589&lt;sup&gt;a&lt;/sup&gt;</td>
<td>740&lt;sup&gt;a&lt;/sup&gt;</td>
<td>476&lt;sup&gt;a&lt;/sup&gt;</td>
<td>523&lt;sup&gt;a&lt;/sup&gt;</td>
<td>360&lt;sup&gt;a&lt;/sup&gt;</td>
<td>549&lt;sup&gt;a&lt;/sup&gt;</td>
<td>281&lt;sup&gt;aa&lt;/sup&gt;</td>
<td>444&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>6 mg Vit B&lt;sub&gt;12&lt;/sub&gt; SC</td>
<td>741</td>
<td>1192&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1690&lt;sup&gt;b&lt;/sup&gt;</td>
<td>720&lt;sup&gt;a&lt;/sup&gt;</td>
<td>688&lt;sup&gt;b&lt;/sup&gt;</td>
<td>486&lt;sup&gt;b&lt;/sup&gt;</td>
<td>765&lt;sup&gt;b&lt;/sup&gt;</td>
<td>454&lt;sup&gt;b&lt;/sup&gt;</td>
<td>826&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>6 mg Vit B&lt;sub&gt;12&lt;/sub&gt; IM</td>
<td>858</td>
<td>1433&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1511&lt;sup&gt;b&lt;/sup&gt;</td>
<td>798&lt;sup&gt;b&lt;/sup&gt;</td>
<td>652&lt;sup&gt;b&lt;/sup&gt;</td>
<td>478&lt;sup&gt;b&lt;/sup&gt;</td>
<td>867&lt;sup&gt;b&lt;/sup&gt;</td>
<td>806&lt;sup&gt;b&lt;/sup&gt;</td>
<td>662&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Trial 2 (1998/99) weaned lambs</td>
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<tr>
<td>Control</td>
<td>370</td>
<td>358&lt;sup&gt;a&lt;/sup&gt;</td>
<td>383&lt;sup&gt;a&lt;/sup&gt;</td>
<td>349&lt;sup&gt;a&lt;/sup&gt;</td>
<td>212&lt;sup&gt;a&lt;/sup&gt;</td>
<td>429&lt;sup&gt;a&lt;/sup&gt;</td>
<td>346&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
<td>589&lt;sup&gt;a&lt;/sup&gt;</td>
<td>517&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>3 mg Vit B&lt;sub&gt;12&lt;/sub&gt;</td>
<td>361</td>
<td>1127&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1334&lt;sup&gt;b&lt;/sup&gt;</td>
<td>840&lt;sup&gt;b&lt;/sup&gt;</td>
<td>420&lt;sup&gt;b&lt;/sup&gt;</td>
<td>540&lt;sup&gt;b&lt;/sup&gt;</td>
<td>473&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-</td>
<td>831&lt;sup&gt;b&lt;/sup&gt;</td>
<td>802&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>6 mg Vit B&lt;sub&gt;12&lt;/sub&gt;</td>
<td>393</td>
<td>2457&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2030&lt;sup&gt;b&lt;/sup&gt;</td>
<td>115&lt;sup&gt;b&lt;/sup&gt;</td>
<td>720&lt;sup&gt;b&lt;/sup&gt;</td>
<td>736&lt;sup&gt;b&lt;/sup&gt;</td>
<td>533&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-</td>
<td>840&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1019&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Trial 3 (1998/99) 4 to 5-week old lambs</td>
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<tr>
<td>Control</td>
<td>71</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>169&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
<td>266&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
<td>72±1.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
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<tr>
<td>6 mg Vit B&lt;sub&gt;12&lt;/sub&gt;</td>
<td>75</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>465&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-</td>
<td>436&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-</td>
<td>200±29.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-</td>
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</tbody>
</table>

Within a column means with different superscripts are significantly different: a v b (P<0.05) A V B (P<0.01)

Table 2. Effect of a long acting injectable Vitamin B<sub>12</sub> and serum (pmol/L) and liver Vitamin B<sub>12</sub> (nmol/kg FW) concentrations in calves (n=10)

<table>
<thead>
<tr>
<th>Day</th>
<th>1</th>
<th>44</th>
<th>110</th>
<th>188</th>
<th>244</th>
<th>1</th>
<th>44</th>
<th>110</th>
<th>188</th>
<th>244</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Serum Vitamin B&lt;sub&gt;12&lt;/sub&gt;</td>
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<td>Liver Vitamin B&lt;sub&gt;12&lt;/sub&gt;</td>
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<td></td>
<td></td>
<td>(day 146)</td>
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<tr>
<td>Control</td>
<td>288</td>
<td>239&lt;sup&gt;a&lt;/sup&gt;</td>
<td>225&lt;sup&gt;aa&lt;/sup&gt;</td>
<td>254</td>
<td>264</td>
<td>459</td>
<td>368&lt;sup&gt;a&lt;/sup&gt;</td>
<td>592&lt;sup&gt;a&lt;/sup&gt;</td>
<td>866</td>
<td>746</td>
</tr>
<tr>
<td>0.12 mg Vit B&lt;sub&gt;12&lt;/sub&gt;/kg LW</td>
<td>332</td>
<td>545&lt;sup&gt;b&lt;/sup&gt;</td>
<td>273&lt;sup&gt;b&lt;/sup&gt;</td>
<td>268</td>
<td>242</td>
<td>440</td>
<td>532&lt;sup&gt;b&lt;/sup&gt;</td>
<td>888&lt;sup&gt;b&lt;/sup&gt;</td>
<td>888</td>
<td>683</td>
</tr>
<tr>
<td>0.18 mg Vit B&lt;sub&gt;12&lt;/sub&gt;/kg LW</td>
<td>335</td>
<td>636&lt;sup&gt;b&lt;/sup&gt;</td>
<td>319&lt;sup&gt;b&lt;/sup&gt;</td>
<td>240</td>
<td>230</td>
<td>438</td>
<td>672&lt;sup&gt;b&lt;/sup&gt;</td>
<td>880&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1013</td>
<td>753</td>
</tr>
<tr>
<td>0.24 mg Vit B&lt;sub&gt;12&lt;/sub&gt;/kg LW</td>
<td>307</td>
<td>632&lt;sup&gt;b&lt;/sup&gt;</td>
<td>383&lt;sup&gt;b&lt;/sup&gt;</td>
<td>287</td>
<td>249</td>
<td>342</td>
<td>640&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1047&lt;sup&gt;b&lt;/sup&gt;</td>
<td>988</td>
<td>640</td>
</tr>
<tr>
<td>0.30 mg Vit B&lt;sub&gt;12&lt;/sub&gt;/kg LW</td>
<td>329</td>
<td>885&lt;sup&gt;b&lt;/sup&gt;</td>
<td>403&lt;sup&gt;b&lt;/sup&gt;</td>
<td>312</td>
<td>324</td>
<td>440</td>
<td>705&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1150&lt;sup&gt;b&lt;/sup&gt;</td>
<td>990</td>
<td>842</td>
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Within a column means with difference superscripts are significantly different a v b (P<0.05) A V B (P<0.01)

**DISCUSSION**

The injectable microencapsulated Vitamin B<sub>12</sub> was effective in increasing and maintaining the serum and liver Vitamin B<sub>12</sub> concentration and the Vitamin B<sub>12</sub> status of lambs and calves for at least 249 and 110 days respectively. New Zealand studies relating changes in serum and liver Vitamin B<sub>12</sub> concentration to growth have demonstrated that lambs with a serum and liver Vitamin B<sub>12</sub> concentration <336 pmol/L and <280 nmol/kg fresh tissue respectively, are Co deficient and these animals will respond in terms of growth rates to Vitamin B<sub>12</sub> supplementation (Clark et al., 1989). Therefore as the four to five-week old lambs were Co deficient, with serum and liver Vitamin B<sub>12</sub> concentrations of 71 pmol/L and 72 nmol/kg fresh tissue respectively, a marked growth response to the injectable microencapsulated Vitamin B<sub>12</sub> was observed.

Cattle are less sensitive to Co deficiency as serum and liver Vitamin B<sub>12</sub> concentration has to be < 73 pmol/L and < 100 nmol/kg fresh tissue respectively before a growth response to Vitamin B<sub>12</sub> supplementation occurs (Clark et al., 1999). The calves were not Co deficient and therefore there was no growth response to Vitamin B<sub>12</sub> supplementation.

The calculated release rate, based on earlier trials, of Vitamin B<sub>12</sub> from a 6 mg depot was 20 µg/day (Grace and Lewis, 1999). Given that the daily Vitamin B<sub>12</sub> required for a 25 kg sheep is 11 µg/day it was not unexpected that a 6 mg Vitamin B<sub>12</sub> injection should
increase and maintain serum and liver Vitamin B₁₂ concentration (Marston, 1970). As a strategy to prevent or treat Co deficiency the injectable microencapsulated Vitamin B₁₂ offers advantages over the intraruminal Co pellets which are the only other long-acting treatment.

The Co deficient lamb can be injected at four weeks of age, whereas the Co pellet must be given to 20 kg lambs that are big enough and have a functional microflora to synthesise Vitamin B₁₂. Giving an injection of Vitamin B₁₂ in the anterior neck region is likely to be quicker and easier than having to carefully position each sheep before the Co pellet is dosed.

REFERENCES


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