The effects of oral drenching with Co or vitamin B_{12}, drenching frequency and Co via rumen bolus on plasma vitamin B_{12} concentration in weaned lambs

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Abstract

Weaned lambs (n=88) were stratified by sex and body weight (BW) and allocated at random to 1 of 10 treatments for 12 weeks (consecutive 6-week periods), to evaluate the effects of supplementation with Co or vitamin B_{12} (**B12**) on the concentrations of B12 in blood plasma. The treatments were: no supplementation (Control); rumen bolus containing Co only (Bolus); and oral administration of a liquid solution (Drench) containing Co or B12 at intervals of 1, 2, 3 or 6 weeks. One bolus (expected Co release [by leaching] of 0.8 mg/day for 4 months) was administered to the bolus group at the initiation of the study (day 0). The lambs on the drench treatments received totals of 63 mg Co (as $CoSO_4 \cdot 7H_2O$) or 34.5 mg B12 over each 6-week period; drench concentrations were 2.1 mg/ml and 2.3 mg/ml for Co and B12, respectively. The lambs were managed in a rotational-grazing system on predominately perennial ryegrass swards. Blood samples were collected weekly (immediately prior to treatment administration), starting on day 0, and plasma was analysed for B12 and Co. Lambs on the Co-drench treatments had higher B12 concentrations ($P < 0.001$) than the Control in both 6-week periods but the difference exhibited a quadratic decline in both periods as the drenching interval increased ($P < 0.001$). Drenching with B12 increased plasma B12 concentration in both periods but the difference only reached significance ($P < 0.05$) in the second period and the effect of drenching frequency was not significant in either period. Lambs on the Co-drench had higher ($P < 0.01$) plasma B12 concentrations than lambs on the B12-drench treatments, and the control. Bolus lambs had a higher plasma B12 concentration ($P < 0.01$) than lambs drenched with Co at 2-, 3- or 6-week intervals in the first 6-week period but did not differ ($P > 0.05$) from these treatments in the second period. Lambs on the Bolus treatment had a higher plasma B12 concentration than Control lambs over the first ($P < 0.001$) and second ($P < 0.05$) periods. It is concluded that drenching with Co is more effective than drenching with B12, in terms of the effect on plasma B12 concentration. Drenching with Co at intervals of 1 or 2 weeks was more effective than drenching at intervals of 3 or 6 weeks. While the bolus treatment was effective in increasing plasma B12 concentration compared to Control over weeks 1 to 12, the difference between these treatments declined significantly over time.

Keywords: Trace minerals, supplementation, method of supplementation, blood, pasture

Introduction

Cobalt is an essential mineral in sheep production (Suttle, 2005) as it is required for the synthesis of vitamin B_{12} (**B12**) by rumen microorganisms (NRC, 2007). Vitamin B12 is involved in propionate metabolism and methionine synthase activity (Kennedy *et al.*, 1992 and Underwood and Suttle, 2001). Subclinical B12 deficiency in sheep results in ill thrift and loss of appetite, which lead to low body weight (BW) gain and eventual BW loss (Robertson, 1971). The concentration of Co in the herbage on many Irish sheep farms is insufficient to meet the dietary requirements of growing lambs (0.2 mg/kg DM; NRC, 2007) thus supplementation is required on many farms (Hession, 2022). Drenching (oral dosing with a liquid) is the most commonly used method in Ireland for mineral supplementation of lambs post weaning (Hession *et al.,* 2022). Previous studies on the effect of frequency of Co drenching of sheep involved animals that were offered Co-deficient diets and exhibited visible signs of clinical deficiency; the drenching was only repeated twice over a ~6 week period (Russel *et al.*, 1975 and MacPherson, 1989) or data were not presented on the effect of supplementation on blood B12 concentration (Lee and Marston, 1969). There is a paucity of data on the effects of repeated drenching with Co on plasma B12 concentration in weaned lambs grazing pasture in temperate climatic regions. Drenches (liquid solution administered orally) containing Co and B12 are commonly used in Ireland (Hession *et al.,* 2022), the UK and other sheep producing countries as supplements for both ewes and lambs. While Marston (1970) reported poor absorption of orally administered B12, Williams *et al.* (2017) reported that a single drench of Co plus B12 (11.5 mg) increased plasma B12 concentration for a period of 7 days, whereas a drench of 3.5, 11.5 or 60 mg Co had no significant effect on plasma B12 concentration but increased plasma Co concentration. The objectives of the current study were to evaluate the effects of: (i) drenching with Co or B12, (ii) the frequency of drenching (intervals of 1, 2, 3 or 6 weeks), and (iii) method of Co administration (pulse delivery given by drench compared with long-term sustained release via a rumen bolus) on blood Co and B12 status of weaned lambs.

Material and methods

All animal procedures used in this study were conducted under approval from the Teagasc Animal Ethics Committee on experimental animal use and experimental license from the Health Products Regulatory Authority (HPRA) in accordance with the European Union protection of animals used for scientific purposes regulations 2012 (S.I. No. 543 of 2012).

Animals and management

Spring-born (March), Charollais-cross weaned lambs (from primiparous Belclare dams; 40 uncastrated males and 48 females; mean initial BW 39.5 [s.d. 5.46] kg) were stratified by sex and BW and allocated to 1 of 10 groups. The 10 groups were then allocated at random to 1 of 10 treatments in a 2 x 4 factorial (Co or B12 drench at intervals of 1, 2, 3 or 6 weeks) + 2 (Co bolus and Control) design on 29 August. The treatments were as follows: no supplementation (Control) (12 lambs); ruminal bolus containing Co only (Bolus) (12 lambs); a Co-only supplement administered by drench at intervals of 1 (Co1), 2 (Co2), 3 (Co3) or 6 (Co6) weeks (8 lambs per treatment); a B12 supplement administered by drench at intervals of 1 (B1), 2 (B2), 3

(B3) or 6 (B6) weeks (8 lambs per treatment). The bolus was of the leaching type and contained Co as $CoSO_4·7H_2O$ (Tracesure Lamb Co., Animax Ltd, Suffolk, UK); it had an expected daily release rate of 0.8 mg Co and an expected efficacy of 4 months. The concentration of Co in the Co-drench was 2.1 mg/ml, as $CoSO_4·7H_2O$ ('Cobalt Super', Chanelle Pharmaceuticals Manufacturing Ltd., Loughrea, Co. Galway, Ireland). The concentration of B12 in the B12-drench was 2.3 mg/ml, as cyanocobalamin (Nettex, Kent, UK). Lambs supplemented at intervals of 1, 2, 3 and 6 weeks received 5, 10, 15 and 30 ml of Co drench at each drenching, respectively, while those on B12 drench received 2.5, 5, 7.5 and 15 ml of B12 drench at each drenching, respectively. Thus, over each 6-week period the drenched animals received the same total amount of either Co (63 mg) or B12 (34.5 mg). All lambs were treated with anthelminthic (ivermectin; Oramec, Merial Animal Health, Harlow, Essex, UK) according to manufacturer's recommendations at the commencement of the study and at 28-day intervals thereafter for the control of gastrointestinal parasites.

The lambs were managed as a single flock in a rotational-grazing system, on a sward of predominantly perennial ryegrass, until October 4 when they were divided by sex and grazed separately but within the same paddock (split using a temporary electric fence). No protein or energy supplementation was offered at any time. All lambs received 2.5 ml of a drench containing 10 mg/ml Co, 200 µg/ml B12 and 0.25 mg/ml Se (Chanelle Cobalt B12; Chanelle Pharmaceuticals Manufacturing Ltd., Loughrea, Co. Galway, Ireland) 26 days prior to the initiation of the study. The lambs grazed to the sward height targets of Keady (2010), with the objective of offering unrestricted access to high quality herbage throughout the study. Lambs were slaughtered on 19 December (28 days after the final drench date).

Measurements

Sward height was recorded, at 50 random locations across the paddocks, immediately prior to grazing and immediately post grazing, using a rising plate meter (Jenquip; Fielding, New Zealand). Pre-grazing samples of herbage were collected throughout the study by taking 20 randomly selected cuts (0.2 m long) to 6 cm above ground level, in a ‘W’ pattern, within each paddock using a handheld clipper (Bahco, SNA, Europe). Disposable gloves were worn to avoid contamination. Samples were stored at -20 °C until processing at which point they were allowed to thaw overnight and then mixed thoroughly prior to selecting a representative sub-sample. The sub-sample was then dried at 40 °C for 48 h in a fan oven (FED 720; Binder GmbH, Tuttlingen, Germany), with moisture extraction facilities, for the determination of DM, and then used for the determination of Co concentration as described by Hession et al., (2021).

Initial BW was recorded on 28 August (the day prior to initiation of the study) and final BW on 18 December. Lambs were slaughtered at an abattoir (European Union approved) that had routine veterinary inspection provided by the Department of Agriculture, Food and the Marine (Dublin, Ireland). Carcass weight was recorded for each lamb and a sample of liver tissue (~100 g) was obtained by excising a narrow slice perpendicular to the liver surface. These samples were frozen and stored at -20 °C until analysed for mineral concentrations as described by Kendall *et al.,* (2015). Lambs were blood sampled weekly, by jugular venepuncture, prior to the administration of oral treatments; samples were collected via 18G x 1 vacuum tube needles (Becton Dickinson and Company, Plymouth, UK) into a 10 ml vacuum tube containing heparin (Becton Dickinson and Company, Plymouth, UK). Tubes were inverted immediately (6 times) before being transported to the laboratory and centrifuged (Sigma 4K15, Harz, Germany) at 2000 g for 15 min. All plasma was removed by pasteur pipette into a 50 mm x 16 mm tube (Sarstedt, Leicester, UK) from which an aliquot (500 µl) was transferred into a 75 mm x 12 mm polypropylene tube (Sarstedt, Leicester, UK) for subsequent B12 analysis. All tubes were capped and stored at -20 °C until analysis. The plasma in the 50 mm x 16 mm tubes was thawed and mixed by rotational agitation (Roller mixer SRT9, Stuart, Staffordshire, UK) before 0.5 ml was pipetted into a 105 mm x 16.5 mm polypropylene tube (Sarstedt, Leicester, UK) prior to analysis for mineral concentrations by inductively coupled plasma mass spectrometry as described by Thomas et al. (2016). The concentration of B12 was determined using a solid-phase, competitive chemiluminescent enzyme immunoassay (CLIA) performed at Axiom Veterinary Laboratories (Devon, UK) (samples from weeks 1, 2, 3, 5, 6, 7, 9, 10 and 11) or CTDS Veterinary Laboratory (Leeds, UK) (samples from weeks 0, 4, 8 and 12) on a Siemens Immulite 2000XPI (used in both laboratories) according to manufacturer's recommendations. Based on their plasma B12 concentration, lambs were classified as being of low (< 188 pmol/L), marginal (188 to 400 pmol/L) or adequate (> 400 pmol/L) B12 status each week (Kendall et al., 2001).

Statistical analysis

Data were analysed using mixed model procedures (Proc MIXED, SAS 9.4). For plasma variables the fixed effects were treatment-by-week and sex, with random terms for dam and lamb. The model for lamb growth and carcass data had fixed effects for treatment, sex and rearing type with dam as a random term. The variation among treatments was partitioned into 1 degree of freedom components, using contrast statements, to evaluate the linear, quadratic and cubic effects of drench frequency for each drench type (Co or B12) and corresponding interactions, the difference between Co drench and B12 drench, the difference between Bolus and Control, and differences among specified subsets of the treatments, including differences from the Control. Dunnett's test was used to evaluate the t-tests that involved comparisons with Control. In the case of plasma Co, a log transformation was required because of heterogeneity of variance across treatment-by-sample time subsets.

Results

The mean Co concentration in the herbage was 0.15 (s.e. 0.024) mg/kg DM (concentrations were 0.14 (s.e. 0.040), 0.12 (s.e. 0.040) and 0.19 (s.e. 0.034) mg/kg DM for Sep, Oct and Nov, respectively). The mean pre- and post-grazing sward heights overall were 8.3 and 4.4 cm, respectively, while the corresponding values between 4 October and the end of the study were 7.9 and 4.2 cm for males and 7.7 and 4.3 cm for females.

Plasma Co concentration

The concentration of Co in plasma on day 0 was 50.9, s.d. 22.53, nmol/L. The effects of treatment on plasma Co concentration for weeks 1 to 12 are presented in Figure 1. Lambs drenched with Co had higher ($P < 0.001$) plasma Co than lambs drenched with B12, averaged over weeks 1 to 12, and there was no effect of drenching frequency. Lambs drenched with B12 had a higher plasma Co concentration than Control when averaged over weeks 1 to 12 (0.15 , s.e. 0.055, ln(nmol/L); $P < 0.05$; Dunnett's test). The mean plasma Co concentration for Control lambs over weeks 1 to 6 of the study was significantly lower than that for lambs on the Bolus treatment (difference = 1.94, s.e. = 0.136 ln(nmol/L); $P < 0.001$) but this largely reflected the very large difference at weeks 1 and 2 since the means for these two treatments converged thereafter as the plasma Co concentration for lambs on the Bolus treatment declined.

Plasma B12 concentration

The concentration of B12 in plasma on day 0 was 102, s.d. 26.3, pmol/L and there were no significant differences among treatment groups. The results are presented for each treatment in Figure 2a and 2b for weeks 1 to 6 and weeks 7 to 12, respectively. Least squares means by main treatments and drenching frequency for weeks 1 to 6 and weeks 7 to 12 are presented in Table 1 and 2, respectively. The results were analysed separately for these two 6-week periods because there was a greater than 2-fold difference in the observed B12 concentration between periods but with no concomitant change in variation. The percentage of lambs on each treatment classified as having low, marginal or adequate B12 concentration each week, between weeks 1 and 6 of the study, is presented in Figure 4a and 4b.

Weeks 1 to 6. Lambs supplemented with Co by drenching had a higher B12 concentration in plasma than lambs drenched with B12 when averaged over weeks 1 to 6 (difference = 139, s.e. 33.2, pmol/L; P < 0.001). There was a downward trend, with significant (P < 0.01) linear and quadratic components, in the average plasma B12 concentration as the drenching interval increased for the Co-drench treatments; there was no evidence for any trend associated with frequency of drenching with B12. On average, 13, 44, 69, 48, 55, 48, 60 and 63% of records for lambs on the Co1, Co2, Co3, Co6, B1, B2, B3 and B6 treatments were classified as having low B12 status over weeks 1 to 6, respectively. The mean B12 concentration was greater than Control for each of the B12 treatments and the difference between Control and the average of all B12 drench treatments was 95, s.e. 45.3, pmol/L (P = 0.05). Plasma B12 concentration did not differ significantly between Bolus treatment and Co1 over weeks 1 to 6 but was significantly greater for the Bolus treatment than for Co2, Co3 and Co6 (P < 0.05, P < 0.01 and P < 0.01, respectively; Dunnett's test). Lambs on the Bolus treatment had higher plasma B12 than the Control group (P < 0.001). Thirteen percent of records from the Bolus treatment were classified as low B12 status on average between weeks 1 and 6, compared with 85% of Control records. The plasma B12 concentrations of Bolus and Control lambs, and the differences between them are presented in Figure 3. The difference between the Bolus and Control declined significantly (P < 0.01) over time.

Weeks 7 to 12. Mean plasma B12 concentration over weeks 7 to 12 was significantly higher for the set of Co drench treatments than for the set of B12 drench treatments (difference = 106, s.e. 33.2 pmol/L; P < 0.01). Plasma concentration of B12 exhibited a significant decline (linear P < 0.01, quadratic P < 0.05) as drenching frequency declined for lambs supplemented with Co but there was no evidence for any change with drenching frequency in the case of the B12 drench. Drenching with Co resulted in higher plasma B12, averaged over weeks 7 to 12, than the Bolus treatment for all drenching frequencies except Co3, and the difference was significant (P < 0.01; Dunnett's test) for Co1. The concentration of B12 in plasma over weeks 7 to 12 was significantly higher for Bolus than Control treatments (P < 0.01). While the mean B12 concentration was higher than Control for each of the B12 treatments none of the differences was significant. However, the average of all B12 drench treatments was significantly higher than Control (difference = 130, s.e. 45.4, pmol/L; P < 0.01). The difference in B12 concentration between these treatments for weeks 7 to 12 was 0.26 ln(nmol/L), s.e. 0.133, which is not significant (P > 0.05).

Animal performance

Lamb performance traits are summarised by treatment in Table 3. There was no evidence for any differential effects of treatment on either lamb growth or carcass weight. The concentration of Co in the liver at slaughter (Table 3) was significantly higher for lambs that were drenched with Co than those drenched with B12 (P < 0.001) and the mean for the B12 treatments was significantly higher than the Control (P < 0.05); no other treatment effects on liver Co concentration were detected.

Discussion

The two primary objectives of the current study were to evaluate the effects of supplementing with Co or B12, and the frequency of supplementation, on plasma Co and B12 status of weaned lambs. The study also involved evaluation of the efficacy of supplying Co by a long-term sustained release system (rumen bolus).

The mean Co concentration of the herbage (0.15 mg/kg DM) was within the range (0.03 to 0.20) previously reported for Irish grassland (Parle *et al.*, 1998) but below current NRC (2007) requirements for growing lambs (0.2 mg/kg DM). The high plasma Co concentration in plasma at day 0 is probably because all lambs received a drench containing 25 mg Co 26 days before the study began. Nevertheless, 89% of the lambs were classified as 'low B12 status' at the initiation of the study.

The increase in B12 concentration in lamb plasma from week 7 onwards was unexpected and cannot be explained by the small increase in Co concentration in herbage during that stage of the study (Oct/Nov). A number of other possible causes of the increase in B12 concentration were ruled out as follows. Firstly, lambs did not have access to any additional Co or B12 source (such as mineral licks, concentrates, or anthelmintics containing Co, etc.) at any stage during the study. Secondly, issues with the analysis of plasma B12 concentration were eliminated as repeat analysis of samples (representing all treatment groups) from week 7 yielded results that were consistent with the initial analysis. Thirdly, the effects of treatment on plasma B12 were quite consistent between the two periods (see Table 2), with no evidence that the magnitude of the increase during the second period was dependent on treatment. Finally, investigation of the within-treatment correlation between weekly values for plasma B12 concentration showed that the average of those representing pairs within each 6-week period did not differ significantly from the average of those that involved weeks from different-period pairs (0.49 v 0.42), indicating that the pattern of animal-to-animal variation was consistent throughout the study.

Supplementation with B12 or Co by drench

In the current study, supplementation with Co increased plasma B12 concentration. Previously Russel *et al.* (1975) reported increased serum B12 concentration in lambs following supplementation with 200 mg cobalt chloride in 15 ml water. MacPherson (1989) reported increased serum B12 concentration in lactating ewes following supplementation with 1, 10, 100 or 250 mg cobalt sulphate. However, Williams *et al.* (2017) observed that a single Co drench of 3.5, 11.5 or 60 mg Co did not significantly increase plasma B12 concentration, over the subsequent 13 days, in housed, weaned lambs offered grass silage (as the sole diet) probably due to the high Co concentration (0.28 mg/kg DM) in the silage.

It is clear that at the doses used in the study, the Co drench was more effective than the B12 drench in terms of the effect on plasma B12 concentration. The information required to calculate the expected effects of these treatments on plasma B12 concentration is not available from the literature. Thus, while Smith and Marston (1970) reported the efficiency associated with converting orally administered Co to B12, by rumen bacteria, and the efficiency of absorption of B12, which depended on whether B12 was orally administered or derived from rumen bacteria, their estimates cannot be applied in the present circumstances since they were all based on data from daily-supplementation regimes. The plasma B12 concentration of lambs supplemented with B12 was higher than Control during both 6-week periods of the current study (the difference averaged over both periods was 112, s.e. 39.3, pmol/L ($P < 0.01$)). The observed increase amounted to 48% of that yielded by drenching with Co and can only be attributed to absorption of orally administered B12.

Frequency of drenching

The decline observed in the concentration of B12 in plasma as the frequency of drenching with Co declined is consistent with the results of Russel *et al.* (1975), Suttle *et al.* (1988) and MacPherson (1989) who reported that the plasma B12 response to Co supplementation was lost after 14 to 21 days. Suttle *et al.* (1988) observed a relatively large increase in plasma B12 concentration following drenching with only 1 mg Co. Russel *et al.* (1975) reported a fall in B12 concentration to pre-drench levels 3 weeks after lambs were drenched with 200 mg cobalt chloride. Similarly, in the current study, drenching at weekly intervals with 10.5 mg Co was more effective in increasing plasma B12 concentration then drenching at intervals of 3 or 6 weeks . However, lambs on the 3- and 6-week Co drench treatments in the present study had higher plasma B12 concentrations than Control lambs for both weeks 1 to 6 and weeks 7 to 12, and the difference averaged over both intervals was significant for both Co3 and Co6 ($P < 0.01$; Dunnett's test).

For the purposes of this discussion, a per-animal definition of the effectiveness of a treatment was defined as plasma B12 concentration > 400 pmol/L on at least 50% of sampling occasions. On this basis, the effectiveness (percent of animals) over weeks 1 to 6 was: 12.5, 12.5, 0 and 12.5% for B1, B2, B3 and B6, respectively, compared with 100, 62.5, 35 and 0% for Co1, Co2, Co3 and Co6, respectively. Hence, on this basis Co drenching is required at weekly intervals to be a fully effective method of preventing Co deficiency, but is labour intensive.

Method of administration

The higher plasma B12 concentration in lambs on the Bolus treatment compared to Control lambs is consistent with the results of Vellema *et al.* (1997), Grace *et al.* (1997) and Kendall *et al.* (2012) who reported increased serum B12 concentrations in sheep following administration of a Co pellet, an eroding controlled release intraruminal device, and Co-containing glass bolus, respectively. In the present study, an initial large increase in plasma B12 concentration following administration of the Co bolus was followed by a steady drop up to week 4 and the difference between Bolus and Control lambs declined steadily throughout the 12 weeks. Previous evaluations of mineral release rate from other boluses administered to ewes (Ritchie *et al.*, 1997) and growing sheep (Hemingway *et al.*, 1997) revealed a high initial daily release rate followed by a lower daily release rate from 6 weeks post administration. These studies involved boluses containing a number of trace minerals and were not directly comparable with the bolus used in the current study. A similar release profile was also observed in sheep administered a Co pellet (Millar and Albyt, 1984).

The estimates of effectiveness were 66.7 and 8.3% for the Bolus and Control, respectively, indicating that the Bolus treatment did not match a weekly Co drench but was comparable in effectiveness to a biweekly Co drench. The B12 drench treatments and drenching with Co at 3 and 6-week intervals were not effective on the basis of this overall assessment criterion.

Liver Co concentration

That the Co drench treatment resulted in a higher liver Co concentration at slaughter than that for the B12 drench treatment is not surprising. Keady *et al.* (2017) also reported an increased liver Co concentration in lambs supplemented with Co. If the Co concentration of B12 is 4.5% (McDowell, 2008), the B12-drench treatments in the current study delivered a much lower total dose of Co than the Co-drench treatments. The finding that lambs on the Bolus treatment did not have a higher liver Co concentration at slaughter relative to control animals reflects the observed plasma Co profile in lambs on the Bolus treatment. As the study progressed, the rate of Co release from the bolus steadily declined as indicated by falling plasma Co concentrations in lambs administered the bolus. Therefore, Co supply was apparently not sufficient during the later phase of the study to increase liver Co concentration. The absence of an effect of drench frequency on liver Co concentration in the current study is probably due to the total Co dose not varying with Co-drench treatment. Whilst the concentration of B12 in liver was not measured in the current study, Marston (1970) reported no significant increase in the concentration of B12 in livers from sheep following 36 weeks of daily oral treatment with 1 or 10 mg of Co.

Conclusion

It is concluded that drenching with Co is more effective at increasing plasma B12 concentration than drenching with B12. Drenching with Co, either weekly or biweekly, resulted in a higher B12 concentration in plasma than drenching at intervals of 3 or 6 weeks. The Co rumen bolus increased plasma B12 concentration but failed to deliver the expected sustained release of Co, thus the elevated B12 concentration observed over the first 6 weeks was not maintained, and therefore a sustained-release leaching bolus may not be an effective method of supplementation over a longer-term period. The general efficacy of sustained-release bolus devices clearly merits evaluation.

CRediT authorship contribution statement

Daniel V. Hession: Conceptualisation, Methodology, Investigation, Data analysis, Writing – original draft, Writing – Review & Editing, Project administration. **Nigel R. Kendall:** Funding acquisition, Conceptualisation, Methodology, Writing – Review & Editing. **James P. Hanrahan:** Data analysis, Writing – Review & Editing. **Tim W.J. Keady:** Funding acquisition, Conceptualisation, Methodology, Writing – Review & Editing.

Declarations of interest: None

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Figure captions

Figure 1 Effect of treatment on plasma Co concentration (log_e (mmol/L). Co1, Co2, Co3, Co6 = Co drench at intervals of 1, 2, 3 and 6 weeks, respectively (total Co = 63 mg in all cases); B1, B2, B3 and B6 = vitamin B12 drench at intervals of 1,2, 3 and 6 weeks, respectively (total vitamin B_{12} = 34.5 mg in all cases); Bolus = intra-ruminal bolus with Co release rate of 0.8 mg/day; Control = no supplement. The concentration of Co in plasma on day 0 was 3.9 (s.d. 0.91) mmol/L. Vertical bars represent s.e.

Figure 2 Effect of treatment on plasma vitamin B_{12} concentration (pmol/L) by treatment: (a) weeks 1 to 6; (b) weeks 7 to 12. Co1, Co2, Co3, Co6 = Co drench at intervals of 1, 2, 3 and 6 weeks, respectively (total Co = 63 mg in all cases); B1, B2, B3 and B6 = vitamin B_{12} drench at intervals of 1,2, 3 and 6 weeks, respectively (total vitamin B12 = 34.5 mg in all cases); Bolus = intra-ruminal bolus with Co release rate of 0.8 mg/day; Control = no supplement. The concentration of vitamin B_{12} in plasma on day 0 was 102 (s.d. 26.3) pmol/L. Vertical bars represent s.e.

Figure 3 Plasma vitamin B_{12} concentration of bolus and control lambs for weeks 1 to 12, and the differences between them fitted with 2^{nd} order trend line. Bolus = intra-ruminal bolus with Co release rate of 0.8 mg/day; Control = no supplement. The concentration of vitamin B_{12} in plasma on day 0 was 102 (s.d. 26.3) pmol/L.

Figure 4 Percentage of lambs with low (<188 pmol/L; Red), marginal (188 – 400 pmol/L; Amber) and adequate (>400 pmol/L; Green) vitamin B_{12} status during weeks 0 to 6 by treatment: a) Co drench and bolus treatments; b) vitamin B_{12} drench and Control treatments. Co1, Co2, Co3, Co6 = Co drench at intervals of 1, 2, 3 and 6 weeks, respectively (total Co = 63 mg in all cases); B1, B2, B3 and B6 = vitamin B_{12} drench at intervals of 1,2, 3 and 6 weeks, respectively (total vitamin B_{12} = 34.5 mg in all cases); Bolus = intra-ruminal bolus with Co release rate of 0.8 mg/day; Control = no supplement.

Table 1 *Least squares means for vitamin B_{12} concentration (pmol/L) in plasma by main treatment, averaged over each 6-week period*

Treatment	Weeks 1 to 6		Weeks 7 to 12	
	Estimate (± s.e.)	P value	Estimate (± s.e.)	P value
Treatment				
Co Drench	386 ± 24.6	-	896 ± 24.7	-
B12 Drench	247 ± 24.5	-	790 ± 24.6	-
Bolus	600 ± 40.1	-	811 ± 40.1	-
Control	152 ± 40.6	-	660 ± 40.7	-
Contrasts				
Co Drench - Control	234 ± 47.4	< 0.001	237 ± 47.5	< 0.001
B12 Drench - Control	96 ± 47.4	n.s.	131 ± 47.5	<0.05
Bolus - Control	448 ± 57.0	< 0.001	152 ± 57.1	< 0.05
Co Drench – B12 Drench	139 ± 34.7	< 0.001	106 ± 34.8	<0.01

[1]Co1, Co2, Co3, Co6 = Co drench at intervals of 1, 2, 3 and 6 weeks, respectively (total Co = 63 mg in all cases); B1, B2, B3 and B6 = vitamin B_{12} drench at intervals of 1,2, 3 and 6 weeks, respectively (total vitamin B_{12} = 34.5 mg in all cases); Bolus = intra-ruminal bolus with Co release rate of 0.8 mg/day; Control = no supplement.

[‡]In the list Co Drench = mean of the 4 Co-drench treatments, likewise for mean of vitamin B12 Drench.

Table 2 *Treatment least squares means (± s.e.) for Drench-by-frequency subclasses in consecutive 6-week treatment periods and the linear and quadratic effects of drench frequency on plasma vitamin B_{12} concentration (pmol/L)*

Treatment[1]	Weeks 1 to 6	Weeks 7 to 12
Co drench		
Co1	573 ± 47.6	1047[a] ± 54.2
Co2	374[a] ± 47.8	923 ± 54.3
Co3	286[a] ± 50.1	794 ± 54.2
Co6	303[a] ± 47.6	823 ± 53.8
Frequency effect		
Linear	***	**
Quadratic	**	*
B_{12} drench		
B1	246 ± 49.7	801 ± 54.1
B2	272 ± 47.5	794 ± 54.0
B3	235 ± 47.6	777 ± 53.8
B6	225 ± 47.6	792 ± 54.0
Frequency effect		
Linear	n.s.	n.s.
Quadratic	n.s.	n.s.
Bolus	600 ± 40.1	811 ± 40.1
Control	152 ± 40.6	660 ± 40.7

[1]Co1, Co2, Co3, Co6 = Co drench at intervals of 1, 2, 3 and 6 weeks, respectively (total Co = 63 mg in all cases); B1, B2, B3 and B6 = vitamin B_{12} drench at intervals of 1,2, 3 and 6 weeks, respectively (total vitamin B_{12} = 34.5 mg in all cases); Bolus = intra-ruminal bolus with Co release rate of 0.8 mg/day; Control = no supplement.

[a]Significantly different from Bolus treatment (P < 0.01; Dunnett's test)

Table 3 *The effects of supplementation with Co or vitamin B_{12}, frequency of drenching and method of administration on lamb performance and liver Co concentration at slaughter*

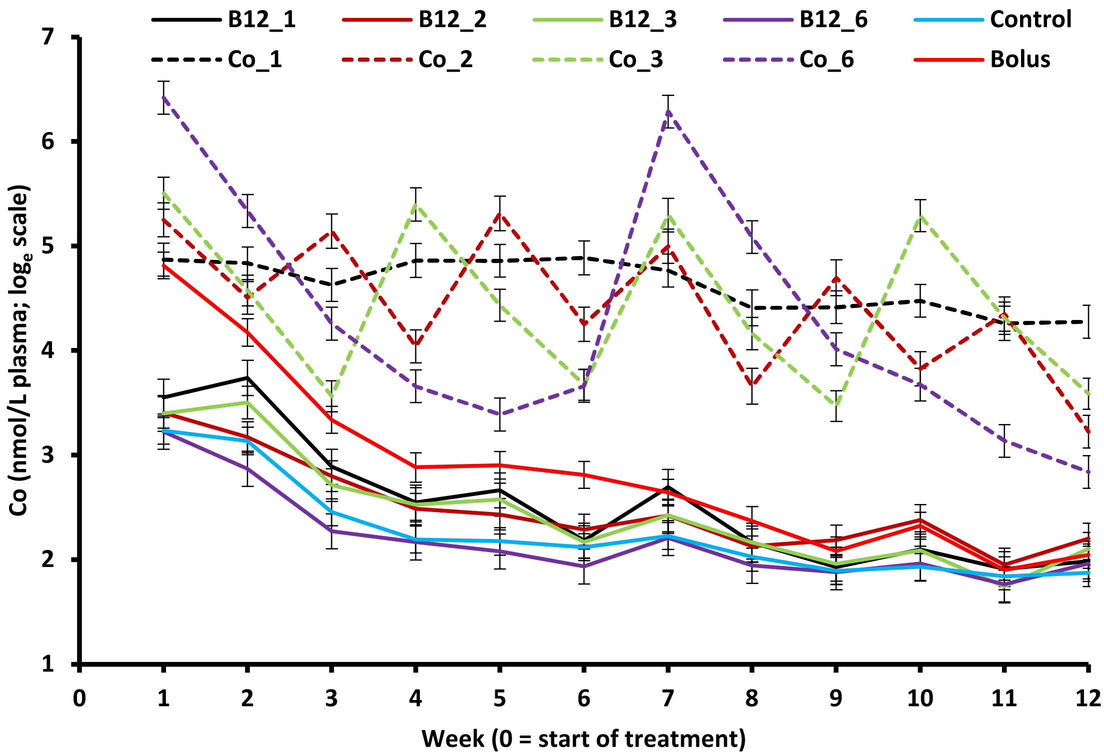
Treatment[1]	Growth rate (g/day)	Final BW (kg)	Carcass weight (kg)	Liver Co[2] (mg/kg DM)
Co drench				
Co1	89 ± 9.9	50.2 ± 0.96	23.9 ± 0.57	5.8 ± 0.45
Co2	77 ± 10.5	49.3 ± 0.99	23.8 ± 0.59	4.2 ± 0.47
Co3	68 ± 9.9	47.5 ± 1.00	23.9 ± 0.59	5.3 ± 0.45
Co6	75 ± 10.4	48.4 ± 1.01	22.5 ± 0.59	5.2 ± 0.47
B_{12} drench				
B1	96 ± 10.7	50.8 ± 1.06	24.1 ± 0.62	2.9 ± 0.49
B2	83 ± 10.5	49.6 ± 1.04	23.7 ± 0.62	3.0 ± 0.49
B3	80 ± 10.2	49.1 ± 1.02	24.1 ± 0.60	3.4 ± 0.47
B6	88 ± 9.9	50.0 ± 1.00	24.1 ± 0.59	2.7 ± 0.45
Bolus	64 ± 8.9	47.6 ± 0.90	23.1 ± 0.53	2.9 ± 0.41
Control	75 ± 9.1	48.8 ± 0.92	23.7 ± 0.54	2.1 ± 0.41

[1]Co1, Co2, Co3, Co6 = Co drench at intervals of 1, 2, 3 and 6 weeks, respectively (total Co = 63 mg in all cases); B1, B2, B3 and B6 = vitamin B_{12}drench at intervals of 1,2, 3 and 6 weeks, respectively (total vitamin B_{12} = 34.5 mg in all cases); Bolus = intra-ruminal bolus with Co release rate of 0.8 mg/day; Control = no supplement.

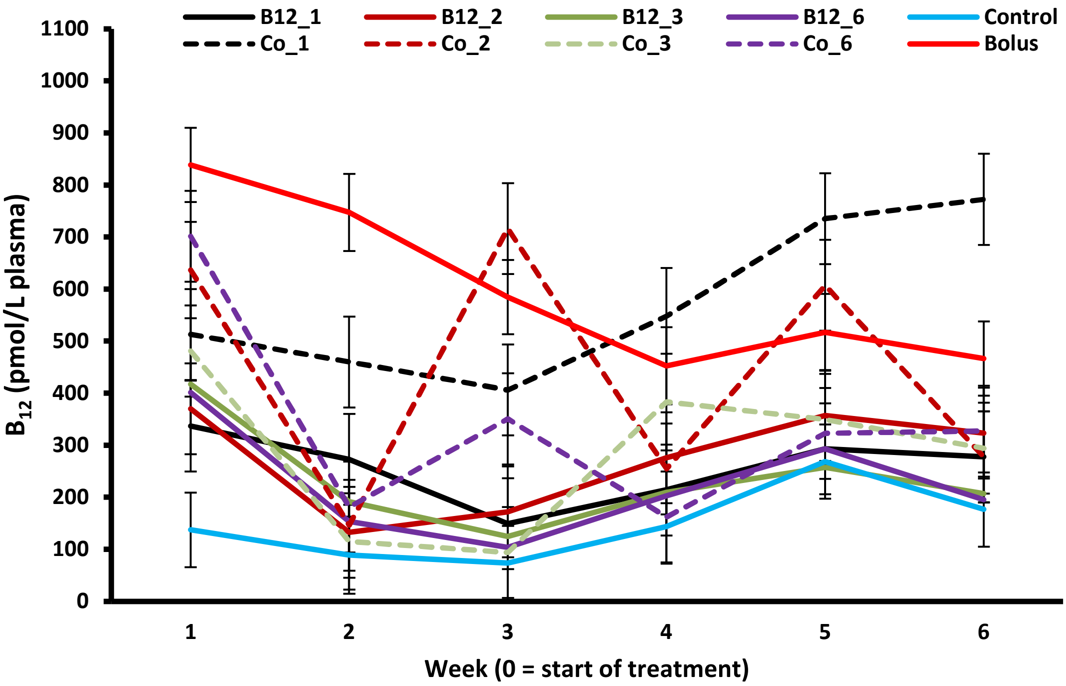
[2]Significant differences between mean of Co-drench treatments and B12-drench treatments ($P < 0.001$) and between the mean of the B12-drench treatments and control

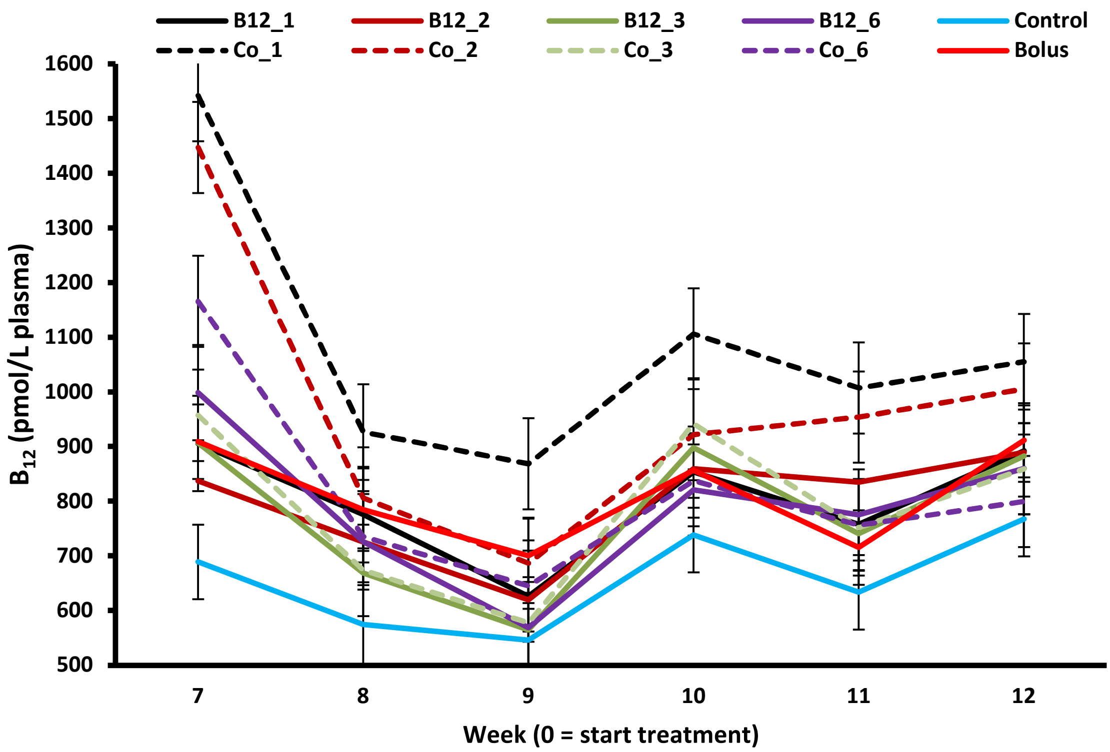
Highlights

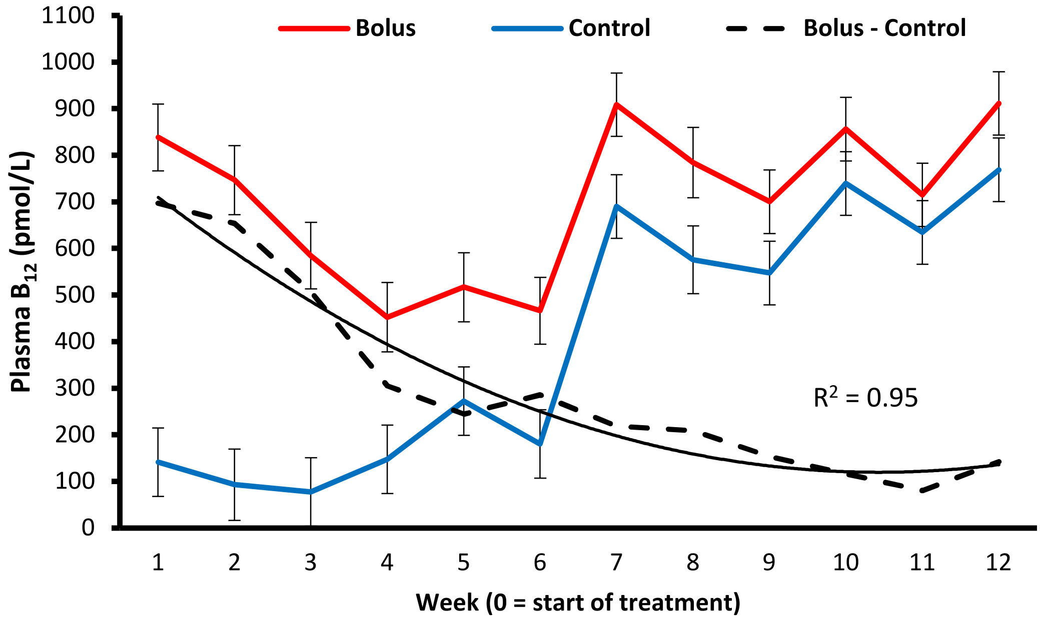
- Drenching with Co is more effective than drenching with vitamin B_{12}
- Weekly drenching with Co maintained adequate vitamin B12 status in weaned lambs
- Weekly drenching is more effective than drenching every 2 weeks
- Rumen bolus failed to deliver the specified sustained release over a 12 week period
- Rumen bolus was as effective over the first 6 weeks as drenching with Co every 2 weeks

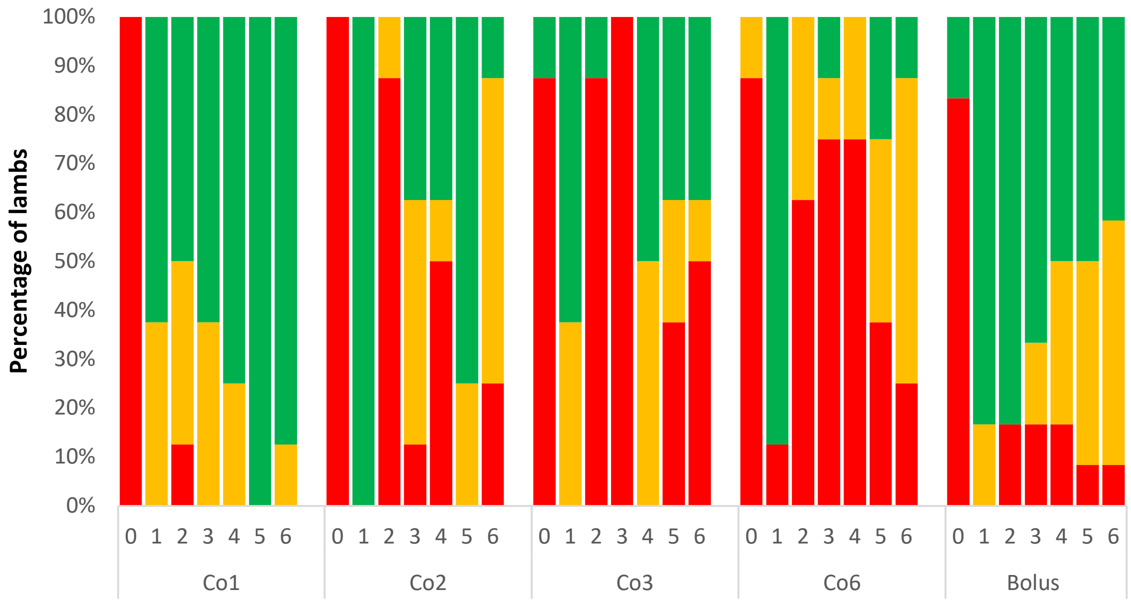


B12_1
B12_2
B12_3
B12_6
Control
Co_1
Co_2
Co_3
Co_6
Bolus
Co (nmol/L plasma; log$_e$ scale)
7
6
5
4
3
2
1
0
1
2
3
4
5
6
7
8
9
10
11
12
Week (0 = start of treatment)

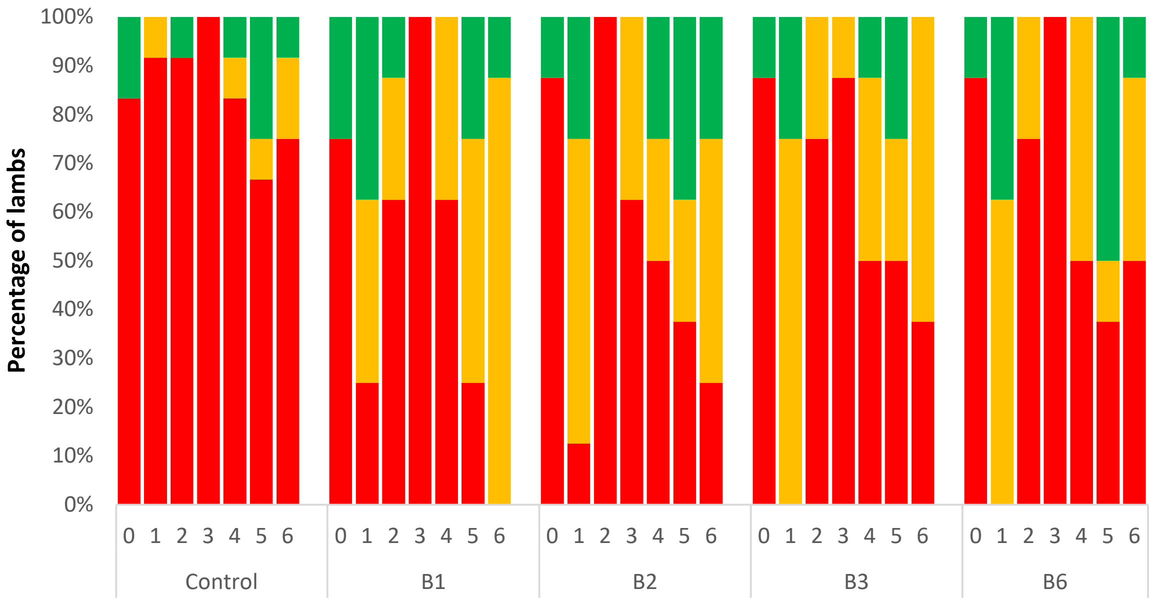

B12_1
B12_2
B12_3
B12_6
Control
Co_1
Co_2
Co_3
Co_6
Bolus
B_{12} (pmol/L plasma)
1100
1000
900
800
700
600
500
400
300
200
100
0
1
2
3
4
5
6
Week (0 = start of treatment)


B12_1
B12_2
B12_3
B12_6
Control
Co_1
Co_2
Co_3
Co_6
Bolus
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
B_{12} (pmol/L plasma)
7
8
9
10
11
12
Week (0 = start treatment)


Bolus
Control
Bolus - Control
Plasma B12 (pmol/L)
1100
1000
900
800
700
600
500
400
300
200
100
0
R2 = 0.95
1
2
3
4
5
6
7
8
9
10
11
12
Week (0 = start of treatment)



Percentage of lambs
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0 1 2 3 4 5 6
Co1
0 1 2 3 4 5 6
Co2
0 1 2 3 4 5 6
Co3
0 1 2 3 4 5 6
Co6
0 1 2 3 4 5 6
Bolus


Percentage of lambs
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0 1 2 3 4 5 6
Control
0 1 2 3 4 5 6
B1
0 1 2 3 4 5 6
B2
0 1 2 3 4 5 6
B3
0 1 2 3 4 5 6
B6