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The effect of parenteral vitamin B₁₂ on the growth rate of dairy calves over the summer and autumn on seven farms from the Central Plateau, New Zealand

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ABSTRACT

Aims: To investigate the effect of parenteral vitamin B₁₂ supplementation on the growth rate of dairy heifer calves over the summer and autumn on seven farms from the Central Plateau of New Zealand, an area historically associated with low cobalt levels in grazing pasture.

Methods: This was a controlled clinical trial conducted on a convenience sample of seven farms with young female calves randomly assigned to three vitamin B₁₂ treatment groups and followed through a grazing season. Two treatment groups received either monthly SC injections of a short-acting (SA) B₁₂ formulation or 3-monthly injections of a long-acting (LA) B₁₂ formulation and the third group received no treatment (NT). No additional parenteral vitamin B₁₂ was given; however, all calves received additional cobalt (0.04–0.4 mg Co/kg liveweight) in the mineralised anthelmintic drenches given orally every month. Liveweight was recorded in December/January and at the end of the trial in May/June/July depending on farm. Pasture cobalt concentrations (mg/kg DM) were measured every month using 500-g herbage samples from 100-m transects in the area about to be grazed by the trial groups.

Results: There was evidence for a difference in growth rate between groups with mean final weight of 228 (95% CI = 212–243) kg for the LA groups, 224 (95% CI = 209–239) kg for the SA groups and 226 (95% CI = 211–241) kg for the NT groups respectively, (global p-value = 0.014). Calves given SA vitamin B₁₂ were 3.77 (95% CI = 0.71–6.82) kg lighter than calves given LA vitamin B₁₂ (p = 0.011). There was no evidence for a change in pasture cobalt concentrations (p = 0.32).

Conclusions and clinical relevance: The results of this trial raise the question as to whether the routine use of vitamin B₁₂ supplementation in young cattle from areas traditionally thought to be cobalt deficient is necessary, and further raise the possibility that vitamin B₁₂ supplementation by repeated injection of SA products may negatively impact growth rates.

Abbreviations: LA: Long-acting; LMER: Linear mixed effects regression; NT: No treatment; SA: Short-acting

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Introduction

As in all other mammals, cattle have a specific requirement for vitamin B₁₂ (cobalamin). However, in weaned cattle and other ruminants (and, probably, camelids; van Saun 2014), the principal source of vitamin B₁₂ is microbial production (Westwood and Laven 2019). Rumen microbes require cobalt to produce vitamin B₁₂, so in healthy cattle, when enough cobalt is fed in the diet there will be sufficient vitamin B₁₂ produced in the rumen to meet the animal's requirements, and when inadequate cobalt is fed, cattle can develop vitamin B₁₂ deficiency.

Vitamin B₁₂ deficiency is a slowly developing disease, with clinical signs becoming apparent only after approximately 6 months of cobalt deficiency (Westwood and Laven 2019). Clinical deficiency is characterised by a gradual loss of appetite, followed by ill-thrift and weight loss, and a range of signs

including cachexia, diarrhoea, listlessness, anaemia, and death (Westwood and Laven 2019). Signs consistent with cobalt deficiency in cattle (eventually named “bush sickness”) were first reported in New Zealand in 1897 (Andrews 1970). It was initially thought to be an iron deficiency, and the condition was treated by supplementing with iron-containing compounds (Reakes and Aston 1919). Results were not consistent, but in the 1930s, the introduction of treatment with limonite (an iron ore consisting of a mixture of hydrated iron oxides and hydroxides) improved response rates, though they still varied depending on the source of the limonite (Andrews 1970). However, in 1934, Filmer and Underwood (1934) showed that limonite was still an effective treatment for “enzootic marasmus” (a Western Australian disease like bush sickness) in cattle even when the iron was removed. In 1935 they concluded that cobalt was the most likely reason for

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this effect (Underwood and Filmer 1935), and in 1937 they demonstrated the value of cobalt supplementation for treating cattle with enzootic marasmus (Filmer and Underwood 1937). Similar studies in New Zealand cattle suffering from bush sickness were not undertaken with the research in New Zealand focusing principally on sheep, which were much more prone to cobalt deficiency (Andrews 1970). Recognition that cobalt deficiency resulted in insufficient production of vitamin B₁₂ led to vitamin B₁₂ supplementation being used to treat cobalt deficiency. Multiple studies have demonstrated the value of vitamin B₁₂ supplementation in pasture-based sheep in New Zealand (Clark *et al.* 1989; Grace *et al.* 2003), but in contrast, no such benefits have been reported in grazing cattle, with both Clark *et al.* (1986, 1999) and Grace *et al.* (2014) reporting no effect of vitamin B₁₂ supplementation on the growth rate of young calves, and Grace and Knowles (2012) reporting no effect of supplementation on lactation. Indeed, Knowles and Grace (2014) stated that cobalt deficiency had not been observed in cattle in New Zealand.

Nevertheless, supplementation of young cattle with vitamin B₁₂ is a common practice in New Zealand (Ellison 2002; Grace and Knowles 2012). This is especially the case in the Central Plateau of the North Island, where supplementation is seen as necessary by farmers and veterinarians alike. The Central Plateau is a volcanic area covering much of the central North Island, extending from the Ruapehu district and Lake Taupō in the south towards Tauranga and the Bay of Plenty in the north-east. The major driver for this use is the low pasture cobalt concentrations in this region (Westwood and Laven 2019), with winter monitoring programmes regularly finding pasture cobalt concentrations below 0.04 mg/kg (VetPlus, data on file), well below the recommended intake of 0.06 mg/kg DM for cattle at pasture (Clark *et al.* 1986; Knowles and Grace 2014).

The aim of this trial was to investigate the effect of parenteral vitamin B₁₂ supplementation on the growth rate of dairy heifer calves over the summer and autumn on seven farms from the Central Plateau of New Zealand.

Materials and methods

Enrolment

All manipulations were approved by the Massey University Animal Ethics Committee, Protocol 17/87.

A convenience sample of seven farms or dairy heifer graziers in the Central Plateau region serviced by VetPlus (Taupō, Waikato, NZ) was selected based on willingness to be involved, a history of parenteral supplementation of B₁₂ for cattle in their first year of grazing, and adequate weighing facilities. All calves on the enrolled farms were crossbred (between 40–60% Friesian and 60–40% Jersey), dairy heifer calves born in July–August (spring) 2017. Under the terms of enrolment, all calves were to be regularly weighed and if the weight difference between treatment groups exceeded 5 kg, or there was evidence of clinical disease, the trial would be discontinued, and feed bought to correct the weight difference.

Treatment groups

In summer, on each of the seven enrolled farms (December 2017 or January 2018, depending on farm), 80, 3–4-month-old, heifer calves were weighed and randomly assigned to three treatment groups. Randomisation was done using the sample function in R (R Core Team 2021, R Foundation for Statistical Computing, Vienna, Austria) to indicate treatment group as the calves lined up in the race, with the probability of selection adjusted so that there was a 25% chance of an animal being allocated to either the “Long-acting” (LA) or “No Treatment” (NT) groups and a 50% chance of being allocated to the “Short-acting” (SA) group. The three treatment groups were: SA (n = 40) 2 mL SC injection of Prolaject vitamin B₁₂ 2000 (Bayer, Auckland, NZ) monthly; LA (n = 20) 1 mL/25 kg SC injection of Smartshot vitamin B₁₂ (Virbac New Zealand Ltd., Hamilton, NZ) at 3-month intervals, and NT (n = 20) no parenteral vitamin B₁₂ supplementation. On all enrolled farms, calves would normally have received monthly injections of SA B₁₂ (Prolaject vitamin B₁₂ 2000), so the NT group was restricted to 20 calves to encourage farmer participation. No additional parenteral vitamin B₁₂ was given; however, all calves received cobalt (0.04–0.4 mg Co/kg liveweight) in a mineralised broad-spectrum anthelmintic containing at least two classes of anthelmintic at a frequency that was always less than the recommended dose interval for that product (see Table 1 for details). Treatment groups were identified using different coloured button tags, so that on-going treatments were correctly allocated, which meant that neither farmers nor veterinary technicians were blinded as

Table 1. Summary of the supply of oral cobalt from the use of mineralised anthelmintics on seven farms from the Central Plateau area of New Zealand during a trial investigating the effect of vitamin B₁₂ injection on the growth of dairy calves at pasture.

Farms	Anthelmintic	Manufacturer	Dose rate	Cobalt dose rate
1, 4	Switch C Hi-mineral	Boehringer Ingelheim	1 mL/20 kg	0.44 mg/kg
3, 5, 6, 7	Matrix C Hi Mineral	Boehringer Ingelheim	1 mL/10 kg	0.44 mg/kg
2	Scanda Selenised	MSD Animal Health	1 mL/10 kg	0.04 mg/kg

to the treatment group membership of individual calves.

Grazing management

For the duration of the trial, calves in all three treatment groups were grazed together on each farm and received the same routine animal health treatments. All calves grazed a ryegrass (*Lolium perenne*)/clover (*Trifolium repens*) mix pasture with a pre-grazing target of 2,800 kg DM/ha grazed to a post-grazing residual of 1,400 kg DM/ha, although with a dry summer (typical in the Central Plateau) pre- and post-grazing targets were decreased. Typically, the calves were checked and moved every second day, onto pasture likely grazed within the last 12 months. Calves were fed only on pasture throughout the study period, with no additional supplement being offered and there were no farms with irrigation.

Data collection

Live weight was recorded at the start of the trial in December/January and at the end in May to July 2018, depending on farm, using Tru-test weigh bars, a Tru-test XR5000 weight indicator and the XRS2 stick reader (Datamars, Auckland, NZ).

During weighing, blood samples were taken from five calves per treatment group either from the coccygeal or the jugular vein into a 10 mL plain Vacutainer (BD Diagnostics, Oxford, UK) using an 18-gauge x 1 inch needle. The same five animals from each treatment group were blood sampled at the beginning and end of the trial. Vitamin B₁₂ status of the study calves was assessed using serum vitamin B₁₂ concentrations (Westwood and Laven 2019). These concentrations were measured by SVS Laboratories (Hamilton, NZ) by electrochemiluminescence competitive immunoassay using a Hitachi Cobas e601 fully automated analyser (Roche Diagnostics NZ Ltd., Auckland, NZ). The normal reference range for serum vitamin B₁₂ concentration for cattle given by SVS Laboratories was 80–231 pmol/L.

Pasture cobalt concentrations were measured every month to assess the level of cobalt in the diet in comparison to the recommended minimum for this stock class of 0.06 mg/kg DM (Clark *et al.* 1986; Knowles and Grace 2014). Herbage samples (approximately 500 g) were harvested along 100-m transects in the area about to be grazed by the trial group and submitted to Hill Laboratories (Hamilton, NZ) for analysis, with the pasture cobalt concentration being estimated using nitric acid/hydrogen peroxide digestion followed by inductively coupled plasma mass spectrometry.

Power analysis

Anonymous weight data from three farms serviced by VetPlus showed that the mean weight gain from December to May for dairy heifers in the previous year was 93.5 (SD = 16.9) kg. Power analysis showed that to detect a weight change of ± 6 kg in treatment calves over control calves with a ratio of two control calves for every treatment animal, then 20 calves in each treatment group and 40 calves in the control group on seven farms would give a power = 0.8, using a design effect of 1.5. The calculation was carried out using the `epi.sscomp` function from the R package `epiR` (Stevenson *et al.* 2022). For this study, the control group were the calves allocated to receive monthly injections of SA vitamin B₁₂ (the usual practice on these farms), and these same 40 calves on each farm would serve as the controls for the 20 calves in each of the other treatment groups, giving a total of 80 calves per farm enrolled.

Statistical analysis

The main research question was whether there were differences in growth rates between the treatment groups. Of secondary interest was whether there were differences in the serum concentration of vitamin B₁₂ between treatment groups and whether pasture cobalt concentration changed during the study. To test the success of the randomisation, it was also important to test whether potential confounders at enrolment, i.e. initial weight, initial age, and initial serum vitamin B₁₂ concentration, were evenly distributed between the treatment groups on each farm. The average pasture cobalt content for each farm during the trial was also calculated. All data analyses were performed in R v4.2.1 (R Core Team 2021, R Foundation for Statistical Computing, Vienna, Austria).

For assessment of confounding, three linear mixed effects regression (LMER) models were built with initial weight, initial age, or initial vitamin B₁₂ concentration as the dependent variable and treatment group as the independent variable, with a random intercept at the farm level.

For assessment of the effect of treatment group on final weight, a LMER model was built with the final live-weight as the dependent variable and treatment group, initial weight, and trial length (days) as the independent variables, with a random intercept at the farm level. The average farm pasture cobalt concentration was also tested in the model and retained at $p < 0.05$ using the log likelihood test.

For assessment of the effect of treatment group on serum vitamin B₁₂ concentration, a LMER model was built with the final serum B₁₂ concentration as the dependent variable and treatment group, initial serum B₁₂ concentration, and trial length (days) as

Table 2. Summary of farm type, enrolment dates and trial events for seven farms from the Central Plateau area of New Zealand enrolled in a study investigating the effect of vitamin B₁₂ injection on the growth of dairy calves at pasture.

Farm identity	Type	Start date	End date	Trial length (days)	Weighing events (n)	Pasture samples (n)
1	Dairy grazier	18/12/2017	24/07/2018	218	2	5
2	Run-off block	6/12/2017	3/05/2018	148	5	5
3	Run-off block	1/12/2017	4/05/2018	154	5	5
4	Dairy grazier	10/01/2018	7/06/2018	148	4	5
5	Dairy grazier	9/01/2018	28/06/2018	170	3	6
6	Run-off block	18/01/2018	3/07/2018	166	6	5
7	Run-off block	18/01/2018	3/07/2018	166	6	5

the independent variables, with a random intercept at the farm level. The average farm pasture cobalt concentration was also tested in the models and retained at $p < 0.05$ using the log likelihood test.

For assessment of the change in pasture cobalt over the trial, a repeated measures random effects model was fitted using the nlme package (Pinheiro *et al.* 2022). The dependent variable was the pasture cobalt concentration, and the independent variable was the days from the earliest pasture sample, with a random intercept at farm level. The covariance function for the residuals from the repeated measure was selected by choosing the model with highest log-likelihood value.

The LSM differences (Lenth 2016) between treatment groups for weight, age, and serum vitamin B₁₂ concentrations at the start of trial, and for weight and serum vitamin B₁₂ concentrations at the end of the study were predicted from the fitted LMER models and tested for significance, with Tukey's method used to control the type 1 error rate arising from multiple comparisons.

Results

Farm characteristics and enrolment dates are summarised in Table 2. On Farms 6 and 7 all weighing, blood sampling and animal treatments were carried out by VetPlus technicians, whilst on the other farms (1–5) the VetPlus technicians did not weigh the calves or administer the SA vitamin B₁₂ injections between the first and last weighing. They did, however, give the second LA B₁₂ injections on these farms. Farm compliance was considered good; technicians or veterinarians when present at weighing events assisted with treatments, the first author reminded clients each month to maintain the trial protocol and the use of

Table 3. Results of linear mixed effects regression models fitted to age, weight, and serum vitamin B₁₂ concentration at start of trial on seven farms from the Central Plateau area of New Zealand. The p-values show that there was no support for a difference in age, weight, and vitamin B₁₂ concentration, respectively, across the three treatment groups.

Variable	n	Mean	Range	P-value
Age at start of trial (days)	524	142.5	86–188	0.75
Weight at start of trial (kg)	559	118.6	77.5–189.0	0.79
Serum B ₁₂ concentration at start of trial (pmol/L)	104	166.4	11.2–533.7	0.53

coloured visual tags in the cattle ears made it easy to identify which treatment group a calf belonged to.

One calf from the SA B₁₂ group on Farm 3 died during the experiment; post-mortem examination by the first author showed that the likely cause of death was polioencephalomalacia. Farm 1 failed to get a vitamin B₁₂ test completed at the end of the trial and the LA vitamin B₁₂ treatment group from Farm 4 did not receive a second injection 3 months after the first.

Assessment of potential confounding at enrolment

There were 35 calves without a birth date: these were removed from the age LMER analysis. The results from the three separate LMER models built to test if age at start of trial, weight at start of trial and B₁₂ concentration at start of trial were potential confounders, showed no evidence for a difference between treatment groups in respect to these variables (Table 3 displays the p-values associated with the inclusion of each variable in the models).

Trial results

Individual farm weight and vitamin B₁₂ results by treatment group are found in Supplementary Table 1.

Final weight

The LMER showed there was no support for an effect of length of trial on final weight ($p = 0.31$), however there was a significant effect of initial weight on final weight ($p < 0.001$) and there were differences in the final weight between the treatment groups controlling for the effect of initial weight ($p = 0.014$). The LSM prediction for final weight was 228 (95% CI = 212–243) kg for the LA group, 224 (95% CI = 209–239) kg for the SA group and 226 (95% CI = 211–241) kg for the NT group. There was evidence that the final weight was 3.77 (95% CI = 0.71–6.82) kg less for the SA than LA groups ($p = 0.011$), but there was no evidence for a difference between the NT and LA groups (difference = -1.85 (95% CI = -5.37 to 1.68) kg; $p = 0.44$), nor between the NT and the SA groups (difference = 1.92 (95% CI = -1.14 to 4.98) kg; $p = 0.30$). There was no support for an effect of mean pasture cobalt on final weight ($p = 0.95$).

Final vitamin B₁₂ serum concentration

Since there was no final B₁₂ sample for herd 1, the analysis was restricted to the other six farms. The LMER showed there was no support for an effect of trial length or initial B₁₂ concentration on the final vitamin B₁₂ serum concentration ($p = 0.88$ and $p = 0.86$, respectively). However, there were differences in the final vitamin B₁₂ concentration between the treatment groups ($p < 0.001$). The LSM prediction for the final vitamin B₁₂ concentration was 261 (95% CI = 219.3–302) pmol/L for the LA group, 203 (95% CI = 161.9–243) pmol/L for the SA group and 136 (95% CI = 95.6–176) pmol/L for the NT group. There was evidence that the final vitamin B₁₂ serum concentration increased 125.1 (95% CI = 63.1–187.2) pmol/L more for the LA than the NT groups ($p < 0.001$) and 66.8 (95% CI = 5.7–128) pmol/L more for the SA than the NT groups ($p = 0.03$), but there was no evidence for a difference between the SA and LA groups (difference = -58.3 (95% CI = -121.0 to 4.3) pmol/L; $p = 0.07$). Furthermore, there was no support for an effect of mean pasture cobalt concentration on the change in vitamin B₁₂ serum concentration ($p = 0.57$).

Pasture cobalt concentrations

The results of the pasture analyses are shown in Figure 1. There were 36 pasture samples from the seven trial farms, with the earliest pasture sample collected on 3 December 2017 and the last on 3 July 2018. The median pasture cobalt content was 0.13 (min 0.02, max 0.49) mg/kg DM and over the whole sampling period 28/36 (78%) pasture samples were above the recommended minimum of 0.06 mg/kg DM, although 6/19 (32%) pasture samples submitted in late summer between January and March had low cobalt concentrations.

An autoregressive covariance function gave the highest log-likelihood value, and the final model found no evidence that the pasture cobalt concentrations (mg/kg DM), changed during the trial ($p = 0.32$).

Discussion

This study on a convenience sample of seven farms in an area of New Zealand known to be at risk of having low soil cobalt and thus low pasture cobalt concentrations, identified an effect of treatment group on final weight ($p = 0.014$ for overall analysis). However, these results cannot be taken to indicate that vitamin B₁₂ supplementation increased final weight in these calves, as the only significant effect found in the individual comparisons was a difference in final weight between calves treated with a LA vitamin B₁₂ supplement and those treated with a SA product. This

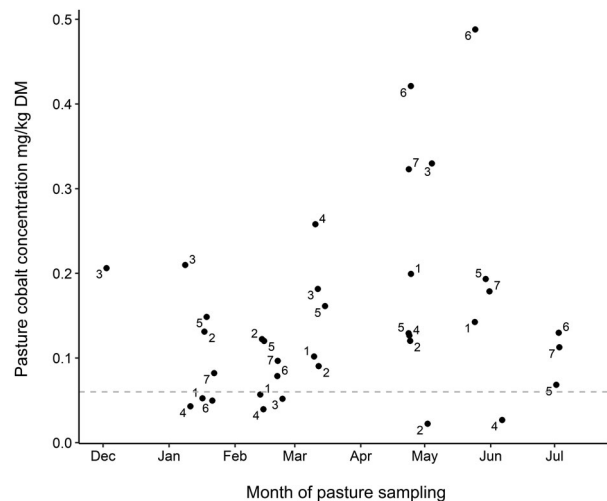


Figure 1. Pasture cobalt concentration (mg/kg DM) by month for seven trial farms in the Central Plateau area of New Zealand enrolled in a trial investigating the effect of vitamin B₁₂ injection on the growth of dairy calves at pasture. The horizontal broken grey line indicates the recommended minimum pasture concentration (0.06 mg/kg DM) and the labels for the data points are the individual farm identifiers.

effect size was small and possibly of little biological significance, with our data suggesting that the likely true difference was between 0.04 and 7.5 kg more growth per calf in the LA group. In contrast, our data were compatible with both positive and negative differences in final weights between the supplemented groups and the NT group, which received no additional vitamin B₁₂ other than the precursor Co present in the mineralised drench,

It is unclear why the SA treatment reduced the final weight compared to the LA treatment. It is unlikely to be an effect of vitamin B₁₂ deficiency, as the serum B₁₂ concentrations in both groups were far higher than the upper threshold of the marginal range (80 pmol/L) suggested by Grace *et al.* (2014). It is possible that the decrease was related to treatment protocol with, for example, pain after the monthly injection reducing grazing behaviour enough to impact weight gain. Although it is tempting to blame the weight difference on the disruption of frequent mustering and yarding, required by monthly injections, this ignores the fact that those calves that were not injected were also mustered and yarded at the same time since they were in the same group. Further research is required to confirm the findings of this study of the difference in weight between the two supplementation groups, and, if the effect is confirmed, to identify the cause.

This study was undertaken on commercial farms which were rearing dairy heifers. As such we did not control variables beyond the vitamin B₁₂ treatment of the study calves. Randomisation was used within each farm to minimise the difference in age, liveweight and serum vitamin B₁₂ concentrations at the start of the study between calves in the three treatment

groups. Our analysis showed that this randomisation process was successful. There were, however, marked differences between farms in the age, liveweight and vitamin B₁₂ concentrations of the calves during the study. This likely reflected differences between farms in pasture cobalt intake, feed management and in their use of supplements prior to the study.

One of the major limitations for this study was that all farms used mineralised anthelmintics, which may have minimised the potential response to additional vitamin B₁₂ supplementation. However, mineral supplementation combined with anthelmintic treatment is commonly used on farms in the Central Plateau and in other areas of New Zealand where cobalt deficiency is thought to occur. This means our lack of benefit is likely to be representative of the response to supplementation on similar farms combining B₁₂ supplementation methods. If anything, this research has highlighted that we do not have any quantitative data on the size and duration of the response to mineral supplementation via anthelmintics in cattle, which means we cannot integrate them into our supplementation regime. This study, using the usual Central Plateau regime, just ignored the contribution from the mineralised drenches which unfortunately means that we are unable to assist practitioners when advising their clients how much they can reduce parental vitamin B₁₂ supplementation if they are using mineralised drenches. Other potential limitations include non-random selection of farms, that results were only presented for a single season, and a lack of monthly weights, which may all have reduced the probability of finding a treatment effect. A further limitation to this study was that most of the pasture samples were above our target cobalt concentration of 0.06 mg/kg DM (Figure 1). In fact, 18/36 (50%) pasture samples had cobalt concentrations greater than the threshold of 0.12 mg/kg DM used to identify a pasture as being potentially deficient for grazing sheep (Suttle 2022). It is likely that these relatively high concentrations of cobalt in the pasture were the key reason why all tested calves on all seven farms had serum vitamin B₁₂ concentrations higher than the upper threshold of the marginal range (80 pmol/L; Grace *et al.* 2014). This meant that rather than being a study of supplementation in calves on a diet that was persistently below requirement, this study was undertaken in calves which for most of the study period, on most farms were grazing pasture containing sufficient cobalt to prevent cobalt deficiency. It is thus unsurprising that we found no effect of vitamin B₁₂ supplementation, when both Clark *et al.* (1986, 1999) and Grace *et al.* (2014) reported no effect of vitamin B₁₂ supplementation on the growth rate of calves fed pasture containing less cobalt than that fed to the calves in the current study. In addition, it must be emphasised that a

threshold of 0.06 mg/kg DM is based on cattle that are performing at or near their potential. Animals that are performing below this level can tolerate cobalt intakes that are much lower than 0.06 mg/kg DM, without becoming cobalt deficient (Suttle 2022).

Conclusion

While we recognise the limitations of the current study, it is likely that there are other farms in areas of New Zealand, where historical practices have led to the routine supplementation of animals with vitamin B₁₂, and where such supplementation is failing to deliver an increase in weight gain. Given the welfare, financial and time costs of repeated injection of cattle, assessment of pasture and animal Co status prior to supplementation should be encouraged.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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