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TITLE OF CASE ***Do not include "a case report"***

THE EFFECT OF COBALT SUPPLEMENTATION ON LAMB GROWTH RATES IN THE FACE OF COBALT DEFICIENCY

SUMMARY ***Up to 150 words summarising the case presentation and outcome (this will be freely available online)***

A hill farm in eastern Scotland, had noted poor lamb growth rates since 2014. Cobalt, selenium and copper deficiencies were reported from historic blood sample results and trace element supplementation had been administered to the ewes, but not the lambs. A supplementation trial was undertaken in 2018 to compare the daily live weight gain (DLWG) between lambs supplemented with trace elements and unsupplemented lambs. The trace element supplements used were intraruminal boluses, containing 51mg cobalt, 10mg selenium and 60mg iodine (Downland Essential Lamb bolus, Downland). Blood samples taken two months post supplementation showed that unsupplemented lambs had cobalt deficient status, but not selenium deficiency. Lambs supplemented with the trace element boluses had an increase in DLWG of 49 g/day compared with unsupplemented lambs. This case confirms that cobalt supplementation on deficient farms can be associated with a significant improvement in growth rates of growing lambs on Scottish hill farms.

BACKGROUND ***Why you think this case is important – why did you write it up?***

In ruminants, rumen microbes incorporate dietary cobalt into vitamin B_{12}, which is released in the abomasum and then absorbed by the small intestine. In the liver vitamin B_{12} forms an essential component of gluconeogenesis from proprionate, a major source of energy in ruminants. Dietary deficiency of cobalt is known to be associated with 'pine', to which sheep are particularly sensitive[1] and growing animals are more susceptible than adults.[2] This production limiting condition is recognised in weaned lambs in many pasture-based sheep systems across the world. Production loss is due to reduced growth rates associated with reduced feed intake;[3] other signs include poor fleece quality, a serous ocular discharge and, in severe cases, anaemia. Ancillary testing of soil, pasture or blood samples can be difficult to interpret,[4] but serum analysis for vitamin B_{12} levels is widely used and interpretation is based on growth-response curves from the 1980s.[5] The purpose of testing should be to assess whether cobalt supplementation would produce sufficient increases in productivity to justify their associated cost, therefore supplementation trials are considered ideal for this purpose.[5]

The investigation described here represents a supplementation trial, undertaken on a hill farm in the east of Scotland. The purpose was to ascertain whether trace element deficiencies were sufficient to interfere with lamb growth rates and what effect the cobalt supplementation had on these lamb growth rates.

CASE PRESENTATION *Presenting features, clinical and environmental history*

This was a hill farm in the east of Scotland with beef cattle and sheep for fat lamb production. There were 450 Scottish Mule ewes, bred to terminal breed (Texel or Suffolk) rams and 400 Scottish Blackface (SBF) ewes bred to SBF or terminal breed rams. The majority of SBF animals were kept on hill grazing for most of the year, except during breeding and lambing time, when they were on improved pasture. The Mule flock was kept on improved pasture and lambed from late March until mid-May. The lambs were sold fat between July and December, with a target live-weight of 39 – 42 kg. Since 2014, there had been a history of poor growth rates in weaned lambs, with cobalt, selenium and copper deficiencies reported from historic blood sample results (data not available). The ewes had been supplemented with these elements in previous years, using 4g copper oxide, 185mg cobalt, 660mg iodine and 100mg selenium boluses (Tracesure, Animax). No supplementation had been used for the lambs as the farm manager was concerned that the benefits may not exceed the cost. However, the growth rate of lambs had been reduced after weaning for at least the previous four years (data not shown here). A trial was undertaken in 2018 to compare the daily live weight gain (DLWG) between lambs supplemented with trace elements (cobalt, selenium and iodine) and unsupplemented lambs.

INVESTIGATIONS *If relevant*

The lambs were kept in several different groups, each group was weighed, and the lambs assigned as supplemented (S) or unsupplemented (U) within a ten-day period (26th June to 5th July 2018). Within each group, lambs were allocated as supplemented (S) or unsupplemented (U) on an alternating basis as they were processed through the weigh scales (not randomised or matched). There were 409 S lambs, each given two boluses containing 51mg cobalt, 10mg selenium and 60mg iodine (Downland Essential Lamb boluses, Downland), and 386 U lambs (Table 1). All lambs included in the trial were born to Mule ewes ('cross-bred' lambs), between 25th March and 1st May 2018. These lambs were kept at grass before and after weaning, with no additional feeding. The lambs were weighed regularly, including at birth, at the time of supplementation, weaning and sale. The lambs were weaned on one of two dates, 355 lambs on 9th July 2018 and 440 lambs on 31th July 2018 (Table 1). Lambs remained in the trial until they were sold for slaughter or until 12th September 2018, whichever came first.

Table 1: Summary of the characteristics of the lambs included in the supplementation trial, with numbers of lambs in each category

		Unsupplemented	Supplemented	Total
Sex	Female	178	203	381
	Male	208	206	414
Litter size	1	41	68	109
	2	283	287	570
	3+	62	54	116
Birthweight	≤4.5 kg	80	71	151
	5.0 - 6.0 kg	242	241	483
	≥6.5 kg	64	97	161
Birth date	< 9th April	212	231	364
	≥ 9th April	174	178	431
Age at treatment (days)	Mean	82	82	82
	Standard deviation	8.7	6.8	7.8
	Minimum	65	63	63
	Maximum	108	98	108

Weaning date	9th July (10–14 weeks old)	175	180	355
	31st July (13–18 weeks old)	211	229	440
Total		409	386	795

The majority of lambs (738 of the 838 cross-bred lambs on farm, 88%) received a routine anthelmintic treatment of ivermectin oral drench, at 0.2 mg/kg bodyweight (0.8 mg/ml, Premadex Drench, Downland), within a seven-day period between 26th July and 2nd August. At other times, 289 separate treatments were given to lambs based on DLWG below 140g/day or faecal staining, as judged by the stockperson.

Blood samples were collected from ten S and ten U lambs, on Monday 27th August. These were collected via jugular venepuncture into 10 ml tubes containing lithium heparin. The serum samples were analysed by SAC Consulting: Veterinary Services, Aberdeen. Ten samples from each group (S and U) were analysed for vitamin B_{12}, using a chemiluminescent enzyme immunoassay, on a immulite XPI (Siemens, Camberley) to assess recent cobalt intake. Six samples from each group were assessed for Glutathione peroxidase (GSH-Px), using a calculation based on a photometric assay run on a RX Imola clinical chemistry system (Randox, London) and a manually performed haematocrit measurement, to assess medium-term selenium intake.

Statistical analysis

Lambs were excluded from the trial if they were provided with supplementary concentrate feed, had birthweights recorded as 10kg or more, or no weight was recorded after supplementation, leaving 795 lambs in the final data set. The data was analysed to determine which factors (Table 2) had most impact on DLWG. The DLWG for each lamb was calculated as

$$Ps = (Wf - Ws) / (Df - Ds)$$

$$Pr = (Ws - Wb) / (Ds - Db)$$

Where *Ps* is DLWG post-supplementation (from the time of supplementation to final weighing), *Wf* is final weight, *Ws* is weight at the time of supplementation, *Df* is date of final weighing and *Ds* is date of supplementation, *Pr* is the DLWG pre-supplementation (from birth to the time of supplementation), *Wb* is birthweight and *Db* is date of birth. The U lambs were weighed on the same dates as supplementation was given to S lambs, therefore this data was used for *Ws* and *Ds* in the U lambs.

StataCorp Version 15, (4905 Lakeway Dr, College Station, TX 77845, USA) was used to select explanatory variables that had a statistically significant impact on post-supplementation DLWG. A linear regression model was used. Relevant explanatory variables were selected for inclusion in the final model at the univariate level ($p<0.2$) and variables were subsequently excluded in a backwards stepwise elimination process ($p<0.05$). For more details see Supplementary materials.

DIFFERENTIAL DIAGNOSIS *If relevant*

TREATMENT ***If relevant***

OUTCOME AND FOLLOW-UP

The mean DLWG was 179g/day in the S group and 130g/day in the U group as shown in Table 3, therefore trace element bolus supplementation was associated with an additional DLWG of 49 g/day for S lambs when compared with U lambs ($p < 0.01$, Table 4 and Figure 1).

Table 3: The DLWG from 795 lambs, 409 of which had been supplemented with Downland Essential Lamb boluses (Downland) and 386 had received no trace element supplementation. Data is taken from the time of bolus supplementation until the final weighing of each lamb (g/day)

Treatment	Mean	Standard deviation	Minimum	Maximum
Unsupplemented	130	67	-67	312
Supplemented	179	48	-38	362
Combined	155	63	-67	362

Table 4: Results of a multivariable linear regression model, showing the variables associated with DLWG (post-supplementation) of 795 lambs on a hill farm in Eastern Scotland in 2018

Variable	Category	Estimate (g/day)	95%CI	P-value
Treatment	Control (ref)			
	Supplemented	49	42 – 57	<0.001
Weaning date	9th July 2018 (ref)			
	31st July 2018	-24	-32 – -16	<0.001

Based on blood sample results (Table 5), S lambs demonstrated higher serum vitamin B_{12} and serum GSH-Px concentrations than U lambs. U lambs had serum vitamin B_{12} levels below the reference range, so would be expected to have a growth rate response to supplementation.[5] Whereas, GSH-Px activity in U and S lambs showed that dietary selenium was adequate in both groups. Lambs remained in the trial until they were sold for slaughter or 12th September 2018, whichever came first.

Table 5: Blood serum results from ten lambs supplemented (S) with trace element boluses and ten lambs not supplemented with trace elements (U). Samples were taken in late August 2018

Treatment group	Serum vitamin B_{12} (pmol/l, ≥295)				Serum Glutathione peroxidase (u/ml, ≥50)			
	Number tested	Minimum	Mean	Maximum	Number tested	Minimum	Mean	Maximum
S	10	149	499.2	>738	6	127.5	172.7	220.6
U	10	<111	119.3	163	6	49.2	86.4	131.9

Estimating the predicted sale price and cost of fattening a lamb post-weaning, gives a reduced profit margin for unsupplemented lambs of £9.62 compared with supplemented lambs (Table 6). For simplicity, post-weaning anthelmintic and other treatments have been assumed to be the same. Also, in the additional 21 days of life post-weaning for the unsupplemented lambs, there is the potential for additional lamb disease or mortality which is not accounted for here, and could increase the difference in net profit margin between supplemented and unsupplemented lambs. This could be especially pertinent given that bolus supplementation of zinc, selenium and cobalt have been linked to increased humoral responses in lambs,[6] which may lead to improved disease resistance and survival rates.

Table 6: Estimation of average costs and sale prices of fattening lambs post-weaning, comparing

unsupplemented and supplemented lambs. Average lamb weaning weights were taken as 30 kg, and weaning date of 9th July, with a target sale weight of 40kg (costs in pounds sterling)

	Unsupplemented lamb		Supplemented lamb	
Downland Essential Lamb bolus (Downland)		-		0.70
DLWG	130g/day		179g/day	
Grazing cost (0.11 per day*)	77 days	8.46	56 days	6.14
Potential sale date and price**	24th September	66	3rd September	74
Sale price minus post-weaning costs		57.54		67.16
Difference		-9.62		Ref

*Cost of grass per kg of dry matter £0.06 – 0.11p,[7] mid-point of £0.09 used in calculations
*Growing lambs eat 4% of their own bodyweight [8]
**Average market sale prices for prime lamb for early and late September were taken from 'Cattle and Sheep Enterprise Profitability in Scotland' [9]

It is possible that the cumulative cost benefit of supplementing lambs is higher than anticipated by the results from this study.

DISCUSSION *Include a very brief review of similar published cases*

On the study farm, an association was found between an increased lamb DLWG of 49 g/day and supplementation with trace element boluses (Downland Essential Lamb bolus, 51mg cobalt, 10mg selenium and 60mg iodine, Downland). Targets for post-weaning DLWG of 200g/day have been suggested,[10] so this represents approximately 25% of the target weight gain. A conservative estimate of the profit margin for fattening a 30kg lamb from weaning to a 40kg sale weight gives an additional £9.62 per lamb for supplemented lambs compared with unsupplemented lambs. Therefore trace element bolus supplementation appeared to be of significant benefit to lamb production on this farm in 2018. The increases in DLWG seen in some studies were greater than in this case, with weight gain advantages of 60 to 180 g/day.[5] However, the degree of deficiency may have been greater in those studies and it is difficult to compare the level of deficiency seen in this case with those in the literature, as many have been determined through different testing methods. Also, the overall nutritional status and breed of the lambs in these studies are likely to have differed from the current case.

Prospective supplementation trials, like the one described here, are considered to be the gold standard for assessing whether cobalt supplementation is financially justifiable.[5] However, these trials can be time consuming, requiring excellent farm facilities and record keeping, and results are not determined for many weeks or months. Therefore, supplementation trials are rarely undertaken and other methods of diagnosing supplement-responsive deficiencies have been sought. Animal blood sampling is most widely used in the UK and reference ranges for serum vitamin B_{12} have been set using growth curves from multiple studies carried out in New Zealand.[5] Nevertheless, serum vitamin B_{12} concentrations must be interpreted with caution, as they respond quickly to changes in dietary cobalt levels and can be elevated by liver damage, poor sample handling or a period of starvation (of just a few hours) prior to sampling.[5] Therefore blood samples should be taken into lithium heparin or plain vacutainer tubes, from lambs at grass without additional feeding, as soon after gathering as possible (at least within four hours). Lamb requirements for cobalt and vitamin B_{12} increase as they become reliant on rumen function for energy, so samples are likely to be representative in lambs over 12 weeks of age and are often taken at weaning for convenience. There is however individual variation in vitamin B_{12} serum concentrations so a minimum of ten samples has been recommended, this can be costly so ensure the client is aware of the cost before proceeding. Liver vitamin B_{12} concentrations are a useful indication of body stores, however few laboratories in the UK can analyse the small samples acquired from sheep liver biopsies. Testing soil for cobalt concentrations is not useful, as the uptake of cobalt from soil varies with plant species, soil pH, soil compaction and the presence of other elements, such as manganese, iron and nickel.[4] Pasture cobalt concentrations can also be complicated by soil contamination, and the rate of conversion of cobalt to vitamin B_{12} does not appear to solely depend on the concentration of cobalt consumed.[11] Accumulation of methylmalonic acid in plasma and urine of cobalt-deficient animals has been noted, but assays are not readily available.[2]

The study farm had the required facilities and willingness to carry out a supplementation trial. Also, despite the historic diagnosis of cobalt, selenium and copper deficiencies from blood samples from sheep on the farm (data not available) and poor post-weaning lamb DLWG, the farm manager was reluctant to supplement all lambs without additional evidence. This was deemed an ideal situation in which to assess the practical benefits of supplementation on a deficient farm.

Mid-season blood samples were also taken from S and U lambs two months after supplementation. These samples could have been taken just one month after supplementation to assess efficacy, but for logistical reasons were delayed in this case. Concentrations of both serum vitamin B_{12} and GSH-Px increased with trace element bolus supplementation, although both U and S lambs demonstrated adequate selenium status. A multi-element bolus was used as multiple deficiencies were suspected. Copper was not included as the benefits to lamb DLWG of copper supplementation in moderate copper deficiencies are considered to be minimal[4] and the risks associated with potential over-supplementation (toxicity) are high.[12] In this case, there appeared to be unnecessary supplementation of selenium. As well as risks of toxicity there is additional product cost associated with unnecessary supplementation. It is also worth ensuring that clients are aware that a few animals will lose boluses shortly after administration. Supplementation options such as cobalt salts or vitamin B_{12} given as oral drenches may be a cost-effective alternative to boluses. However, body storage of cobalt (as vitamin B_{12}) is low and one-off administrations might only provide short term benefits, dependent on the degree of deficiency.[4] Based on this, the additional labour costs of repeat administrations may negate any cost savings. There is also some recent data suggesting that one-off oral drenches with cobalt may not elevate serum vitamin B_{12} levels significantly,[13] however in this study the background diet was not cobalt deficient therefore the clinical effect could not be assessed.

Other forms of supplementation include short or long acting vitamin B_{12} injections. The soluble short acting vitamin B_{12} injections need repeat applications,[14] like the oral drenches, and so incur high labour costs. Nevertheless, these short acting oral or injectable forms are a cost-effective way of maintaining growth rates in short-keep lambs nearly ready for slaughter and are useful for lambs too small for bolus administration. Long acting, deposition injections have been developed, can be imported from New Zealand,[15] and are becoming popular for supplementation. There is also the option to treat pasture with various forms of cobalt.[4] Associated supplement, fuel and machinery costs can be prohibitive, and animal testing is required to determine which parts of the farm consistently need supplementation, but in the author's experience farmers are often satisfied with the results. Free-access minerals, such as powders or blocks, are readily available but are expensive and the consumption rate is thought to vary between individual animals.[16] Trace minerals are also incorporated into commercially available concentrate feeds, but this is an expensive source of supplementation. In this case, the farm manager opted to continue using multi-element boluses, as the response to these had been observed by the farm staff and the required labour input was low.

Due to the variable nature of cobalt and selenium deficiencies from year to year,[5] blood testing of unsupplemented and supplemented lambs after weaning is recommended to monitor the requirement for and efficacy of supplementation.[4] This case demonstrates the importance of investigating trace element deficiencies on farms with low post-weaning DLWG in lambs. The potential increase in DLWG from supplementation is significant, with associated increases in profit margins for fat lamb producers. In addition, serum vitamin B_{12} analysis matched the growth-response outcome in this trial, increasing confidence in the use of this diagnostic test in Scottish hill farms.

LEARNING POINTS/TAKE HOME MESSAGES ***3 to 5 bullet points – this is a required field***

- Supplementation trials are the gold standard method of assessing whether trace element supplementation in lambs is necessary
- Supplementation trials require good on-farm facilities and record keeping
- Supplementation of cobalt in lambs with low vitamin B_{12} serum levels has been shown to be beneficial to growth rates on this study farm

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FIGURE/VIDEO CAPTIONS ***figures should NOT be embedded in this document***

Figure 1: Post-supplementation daily live weight gain in supplemented (S) or unsupplemented (U) lambs. The box and whisper plot shows the median (horizontal line within box), mean (X), upper and lower quartiles and outliers (dots).

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