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Effect of a copper, cobalt and selenium soluble glass bolus given to grazing sheep

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Abstract

Three field trials were carried out to evaluate the performance of a sintered soluble glass copper, cobalt and selenium bolus for maintaining adequate levels of the three trace elements. Trial 1 used 34 growing lambs, trial 2 used 36 growing lambs whilst trial 3 used 50 year-old non productive female sheep. In each trial half the sheep had a bolus administered (bolused) and half remained as controls. Blood samples were taken immediately prior to bolus administration and then at regular intervals throughout each trial. The samples were analysed for copper status (serum caeruloplasmin activity and plasma copper concentration), cobalt status (serum vitamin B_{12} concentration) and selenium status (erythrocyte glutathione peroxidase activity). For trial 1 the bolused sheep at all post treatment samplings (days 20, 42 and 63) were significantly increased in both cobalt and selenium status ($P < 0.001$) compared to the controls, however, there were no significant differences in any other blood parameter. For trial 1 liver copper concentrations were analysed on slaughter samples and were significantly increased for the bolused lambs ($P < 0.001$). In trial 2 the bolused sheep had significantly increased selenium and cobalt status ($P < 0.001$) for all samples (days 28, 51, 69, 91). In trial 3 the selenium status of the bolused sheep was significantly increased at all three samplings (day 21, $P < 0.05$ and days 51 and 105, $P < 0.001$), whilst the cobalt status was also significantly increased on all sample days (day 21, $P < 0.05$; day 51, $P < 0.01$ and day 105 $P < 0.001$). The sintered soluble glass copper, cobalt and selenium bolus was able to prevent or correct deficient and/or marginal cobalt and selenium status of sheep throughout these trials. The bolus had little measured effect on the already adequate blood parameters of copper status, although the liver copper concentrations of the bolused sheep were higher in the trial for which they were analysed.

Keywords: Trace elements; Controlled release; Supplementation; Ruminants

1. Introduction

Trace element deficiencies are common in ruminants, especially extensively grazed animals with

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access only to grass and other naturally occurring plant material as their staple diet (McDowell, 1992; Lee et al., 1999). In the extensive situation, supplementing animals with trace elements can be difficult. Using supplemental feed as a trace element carrier incurs the costs of both feed and labour, if additional feed is not required. Free access minerals, mineral licks and blocks are subject to variable intakes with animals consuming between nothing and many times the required intake (McDowell, 1992). Oral dosing with trace element drenches is another possible alternative. Although this ensures that each animal receives a dose, it may need regular handling, storage mechanisms for the element and/or a high animal tolerance to the levels of element given for long term dosing. Previous work has shown oral drenches to be effective for only short periods in the correction of clinical copper deficiency (1–2 weeks) (Kendall et al., 2000). The controlled release bolus route should provide each animal with a consistent dose in line with its requirements sustained over a long period of time, such that one treatment of the animals should ensure adequate trace element cover for a number of months.

In the early 1980s, Pilkington PLC produced a soluble glass bolus containing copper, cobalt and selenium (Cosecure) designed to dissolve over an 18-month period. This bolus contained copper, cobalt and selenium as part of a sodium phosphate based glass with all the ingredients melted together then cast into a solid (monolithic) glass bolus (Knott et al., 1985). This type of bolus gave excellent results in field trials and has been previously shown to correct deficiencies of copper, cobalt and selenium in extensively grazed ewes over a 345-day period (Telfer et al., 1984). Trengrove and Judson (1985) showed effective release of copper from this type of bolus for 51 weeks. They measured significantly increased liver copper concentrations throughout the trial and whilst the plasma copper concentrations were significantly higher than for the controls during the first 17 weeks they exhibited no significant difference after this, when compared to the adequate control values. McFarlane et al. (1991) found this type of bolus to raise and maintain elevated liver, plasma, blood and cell copper concentrations for 55 weeks, whilst they also demonstrated raised blood and liver selenium concentrations, all within the normal physiological ranges. However, there were some problems encountered in the manufacture of this form of bolus. Investigation by the manufacturers showed that some batches of glass had variable dissolution properties (very fast to zero dissolution) due to temperature fluctuations in the melter furnace. These problems were encountered by MacPherson (1985) during a trial investigating methods of copper supplementation, in which they found that in a group of sheep, the boluses administered performed only marginally better than the control lambs. Subsequently, MacPherson and Gray (1985) reported that the bolused sheep had a 10% incidence of swayback. These results indicated a reduced solubility of batches of monolithic soluble glass boluses. Therefore a new manufacturing process was designed and introduced which produced a (sintered) soluble glass bolus with more consistent release properties. This sintered form of bolus (Cosecure, Telsol) has different dissolution characteristics compared to the original monolithic bolus and therefore requires confirmation of its efficacy and duration of action in the field.

In the trials described here we are using the sintered form of soluble glass bolus to supply copper, cobalt and selenium to extensively grazed sheep in two different situations: lowland finishing lambs and upland sheep in the non-productive year between being a lamb and being a productive ewe (gimmer).

2. Materials and methods

The new sintered type of bolus has had the manufacturing process altered from the monolithic type of bolus process by removing selenium from the basic glass and producing a specific copper–cobalt–sodium–magnesium–polyphosphate glass. This glass is then ground into a fine powder, selenium in the form of sodium selenate added and the homogenous mix formed into a bolus which is then heated (360°C) and annealed to produce a (sintered) glass bolus. The sintered copper, cobalt and selenium soluble glass boluses were formulated to contain (w/w) 13.4% copper (4.7 g), 0.5% cobalt (0.18 g) and 0.15% selenium (0.05 g), (Cosecure®, Telsol).

Three separate trials were carried out over the

summer grazing period. Trials 1 and 2 were carried out on the lowland University of Leeds Farm, using 34 and 36 growing Suffolk and Texel crossbred lambs, respectively. Trial 3 was carried out in the North East of Scotland on upland pasture and used 50 North Country Cheviot gimmers (year old females). The sheep were split into two groups on day 0, by restricted randomisation of liveweight. One group was bolused with a 35 g copper, cobalt and selenium containing soluble glass bolus (Cosecure) and the other group left unbolused as a control group. The sheep were blood sampled by jugular venipuncture on day 0 and subsequently at regular times until most of the lambs were slaughtered (trials 1 and 2) or 105 days (trial 3). The blood samples were analysed for copper status [serum caeruloplasmin activity (CP) (Henry et al., 1974, adapted for the Cobas mira, Roche) and plasma copper concentration (PlCu) (Kendall and Telfer, 2000)], cobalt status [serum vitamin B_{12} concentration (B_{12}) (Radioassay, ICN)] and selenium status [erythrocyte glutathione peroxidase activity (GSHPx) (Paglia and Valentine, 1967, adapted for the Cobas mira, Roche)]. Liver samples were also collected from trial 1 when lambs were slaughtered at either day 86 or day 121 and these were analysed for their copper and zinc concentrations (Kendall et al., 1997). Boluses were recovered from lambs at slaughter for trials 1 and 2. Bolus dissolution rates were calculated by dividing the difference between the administered and recovered bolus weights by the days between recovery and administration. Where more than one bolus was recovered at a specified time the results are expressed as mean and standard error of the mean (SEM).

Results of erythrocyte glutathione peroxidase activities and serum vitamin B_{12} concentrations and were classified according to status categories, as described by Anderson (1981) and MacPherson (1982), respectively. Plasma copper distributions were described according to the Veterinary Laboratories Agency (VLA) criteria and the distribution of caeruloplasmin according to Henry et al. (1974).

The distributions according to treatment and trace element status category were analysed statistically using Chi square analysis. Statistical analysis of liver copper was carried out by ANOVA using GLM on MINITAB 11 with blocking for slaughter date and regression analysis which was carried out using EXCEL 97. All other statistical analysis was carried out using ANOVA with day 0 as a covariate using GLM on MINITAB 11.

3. Results

The erythrocyte glutathione peroxidase activities and serum vitamin B_{12} concentrations for trials 1 and 2 were all significantly increased for the bolused sheep ($P < 0.001$) on all sampling days (Figs. 1–4).

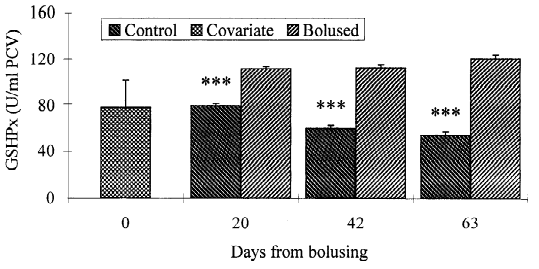

Fig. 1. Mean erythrocyte glutathione peroxidase activities (SEM) of the sheep in trial 1.

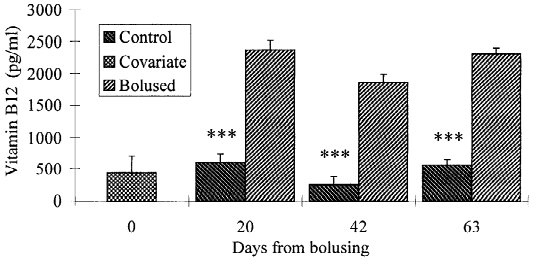

Fig. 2. Mean serum vitamin B_{12} concentrations (SEM) of the sheep in trial 1.

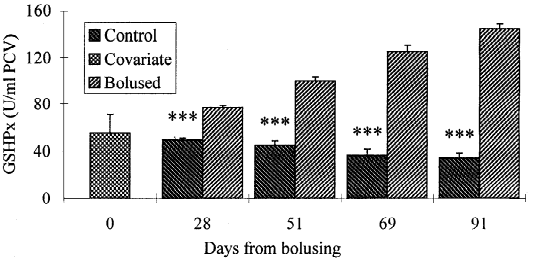

Fig. 3. Mean erythrocyte glutathione peroxidase activities (SEM) of the sheep in trial 2.

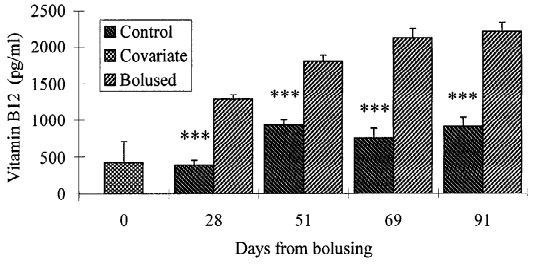

Fig. 4. Mean serum vitamin B_{12} concentrations (SEM) of the sheep in trial 2.

For trial 3, the erythrocyte glutathione peroxidase activities were significantly higher for the bolused sheep on days 21 ($P < 0.05$), 51 and 105 ($P < 0.001$) (Fig. 5). The serum vitamin B_{12} concentrations were significantly higher for the bolused group on days 21 ($P < 0.05$), 51 ($P < 0.01$) and 105 ($P < 0.001$) (Fig. 6).

The liver copper concentrations were significantly higher for the bolused group ($P < 0.001$) in trial 1, the only trial for which they were measured (Table 1). The bolused group had a range of 145–882 mg Cu/kg liver DM, whilst the control group range was

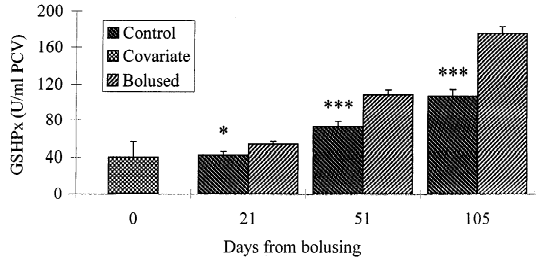

Fig. 5. Mean erythrocyte glutathione peroxidase activities (SEM) of the sheep in trial 3.

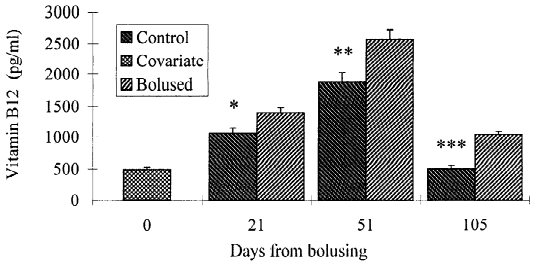

Fig. 6. Mean serum vitamin B_{12} concentrations (SEM) of the sheep in trial 3.

25–310 mg Cu/kg liver DM. The plasma copper concentrations and caeruloplasmin activities in trials 1 and 2 indicated an adequate mean copper status for all groups at all samplings with no significance between the bolused and control sheep (Tables 1 and 2). The copper status indicators for trial 3 (Table 3) indicate an adequate plasma copper concentration, although on day 51 the caeruloplasmin activities were low (deficiency < 15 mg/dl). There were no significant differences between the liveweights of the two groups in trials 1 and 2 (Tables 1 and 2), whilst trial 3 was carried out with sheep in a non-growth phase.

Boluses were recovered from all 17 bolused sheep in trial 1. One bolus was recovered at day 30 from a sheep that died (102 mg/day), seven at the day 86 slaughtering (115 mg/day, SEM 18.9) and nine at the day 121 slaughtering (126 mg/day, SEM 18.7). In trial 2 all attempted recoveries were successful with four boluses recovered at slaughter on day 93 (154 mg/day, SEM 26) and six boluses at day slaughter on 107 (111 mg/day, SEM 19.2), another bolus was recovered at day 3 from a lamb that died (152 mg/day). No boluses were recovered in trial 3, as these sheep were not slaughtered. The overall mean dissolution rate of recovered boluses was 126 mg glass/day (SEM 9.6) which equates to a mean daily release of 16.9 mg Cu (SEM 1.30), 0.63 mg Co (SEM 0.049) and 0.20 mg Se (SEM 0.015).

The distributions of the sheep within the various trace element parameters are shown in Tables 4–6.

4. Discussion

The bolus dissolution rates ranged from 69 to 249 mg glass/day with a mean dissolution rate of 126 mg/day (SEM 9.6). At this rate the boluses would have lasted for between 141 and 507 days with a mean of 278 days (SEM 21.3). The solubility of the glass over the normal rumen pH range (5.5–6.5) is 3 mg glass dissolving per cm^2 of bolus surface area per 24 h (Telsol) which gives a calculated release rate from a virgin bolus of 90 mg/glass per day. However, compared to the original monolithic bolus, the sintered bolus develops a rough surface during dissolution resulting in an increased surface area with time and hence a more rapid dissolution. The

Table 1
Results of copper and liveweight parameters for trial 1

Copper parameter	Day	Control mean	Bolused mean	SEM (S.D)	Probability
Plasma copper	0	14.5		(2.9)	
concentration	20	18.7	19.5	0.8	NS
(μM)	42	17.8	16.9	0.7	NS
	63	16.3	13.5	1	NS
Caeruloplasmin	0	24.7		(6.8)	
activity	20	30.0	31.3	2.2	NS
(mg/dl)	42	29.1	27.9	1.7	NS
	63	29.2	24.5	2.0	NS
Liver copper (g/kg DM)	86/121	153	466	38	$P<0.001$
Live weight	0	27.9		(3.7)	
(kg)	20	33.4	32.5	0.4	NS
	42	35.8	35.6	0.5	NS
	63	36.4	37.2	0.8	NS

particle size of the selenium source is very important in controlling the bolus dissolution rates. Much of the variability in bolus dissolution rates in these trials was due to inconsistent particle size, with the larger particles of selenium leading to a greater than expected increase in bolus surface area. Subsequent to these trials a more consistent grain size of sodium selenate has been used in the manufacture of the sintered bolus and this has reduced variability in the dissolution rates of the bolus (Telsol/ADAS trials, personal communication).

The bolus provided an average of 11.3 mg Cu/kg DM (range 6.2–22.2), 0.42 mg Co/kg DM (range 0.23–0.83) and 0.13 mg Se/kg DM (range 0.07–0.25). In each trial the expected dry matter intake of each sheep was ≈ 1.5 kg/sheep day. When compared to the NRC (1985) allowances (7–11 mg Cu/kg DM for a molybdenum content of the diet of

Table 2
Results of the copper and liveweight parameters for trial 2

Copper parameter	Day	Control mean	Bolused mean	SEM (S.D)	Probability
Plasma copper	0	18.8		(2.6)	
concentration	28	15.6	14.6	0.7	NS
(μM)	51	14.9	16.5	0.7	NS
	69	15.5	15.1	0.7	NS
	91	14.3	14.5	0.6	NS
Caeruloplasmin	0	35.5		(6.2)	
activity	28	28.1	25.7	1.7	NS
(mg/dl)	51	24.2	28.4	1.6	NS
	69	30.4	31.3	2.2	NS
	91	24.7	26.1	1.4	NS
Live weight	0	24.8		(3.4)	
(kg)	28	27.6	28.2	0.7	NS
	51	30.5	30.4	0.5	NS
	69	32.6	31.9	0.5	NS
	91	34.9	34.5	0.6	NS

Table 3
Results of the copper parameters for trial 3

Copper parameter	Day	Control mean	Bolused mean	SEM (S.D)	Probability
Plasma	0	12.9		(1.8)	
copper	21	12.7	13.4	0.4	NS
concentration	51	13.9	13.6	0.6	NS
(μM)	105	15.9	16.3	0.5	NS
Caeruloplasmin	0	23.8		(5.5)	
activity	21	29.0	31.2	1.5	NS
(mg/dl)	51	18.8	18.6	1.0	NS
	105	27.6	29.4	1.4	NS

<0.1 mg/kg DM, 0.1 mg Co/kg DM and 0.1–0.2 mg Se/kg DM), the boluses released amounts sufficient to satisfy the majority of the daily requirements of the sheep for all three elements. However, all of these trace element doses, including the maximum dissolution rate, are less than the NRC (1985) maximum tolerable levels of 25, 10 and 2 mg/kg DM for Cu, Co and Se, respectively.

The supplementation of the already adequate levels of copper (seen in the control group) with a further daily allowance had no effect on the copper parameters except to increase the liver copper concentration that was measured in trial 1. This is consistent with findings of Trengrove and Judson (1985) who found that when supplementing sheep with the monolithic type of bolus, the bolus did not further raise the already adequate plasma copper concentrations, although it did consistently raise the liver copper concentrations. The increase in liver copper concentrations correlated with the bolus release rate of copper (r^2 0.82). No other parameter in either trial 1 or 2 was found to correlate with the bolus release rate. The additional copper did not induce clinical copper toxicity in any sheep in any of these trials. Three sheep in trial 1 had boluses dissolving at a rate faster than expected (240 day

Table 4
Distribution of cobalt, selenium and copper status in trial 1

	Range	Status	Day 0		Day 28		Day 51		Day 69		Day 91	
			Bolus	Control	Bolus	Control	Bolus	Control	Bolus	Control	Bolus	Control
Vitamin	<200	Deficient	1	3	0	1	0	0	0	1	0	0
B_{12}	200–400	Marginal	7	8	0	9	0	1	0	0	0	1
(pg/dl)	>400	Adequate	10	7	17	8	17	16	17	16	17	16
	$P=$			0.95		0.04		0.98		0.98		0.98
GSHPx	<13.5	Deficient	0	0	0	0	0	0	0	1	0	0
(U/ml	13.5–28		0	0	0	1	0	1	0	5	0	4
PCV)	28–42		2	4	0	6	0	9	0	5	0	9
	>42	Adequate	16	14	17	11	17	8	17	7	17	5
	$P=$			1.00		0.41		0.10		0.06		0.01
PlCu	<9.3	Deficient	0	0	0	0	0	0	0	0	0	0
(μM)	9.3–12	Marginal	0	0	0	2	0	2	0	2	4	3
	12–23	Normal	15	18	16	15	17	16	17	15	13	15
	>23	High	3	0	1	1	0	0	0	1	0	0
	$P=$			0.92		0.98		0.98		0.93		1.00
CP	<15	Deficient	0	0	0	1	0	0	0	0	0	0
(mg/dl)	>15	Adequate	18	18	17	17	17	18	17	18	17	18
	$P=$			0.87		0.98		0.87		0.87		0.87

Table 5
Distribution of cobalt, selenium and copper status in trial 2

	Range	Status	Day 0		Day 20		Day 42		Day 63	
			Bolused	Control	Bolused	Control	Bolused	Control	Bolused	Control
Vitamin	<200	Deficient	1	2	0	0	0	7	0	0
B_{12}	200–400	Marginal	7	8	0	9	0	8	0	5
(pg/dl)	>400	Adequate	9	7	17	8	16	2	16	12
	$P=$			1.00		0.06		<0.001		0.48
GSHPx	13.5	Deficient	0	0	0	0	0	0	0	0
(U/ml	13.5–28		0	0	0	0	0	0	0	0
PCV)	28–42		0	0	0	0	0	2	0	5
	>42	Adequate	17	17	17	17	16	15	16	12
	$P=$			1.00		1.00		0.98		0.70
PlCu	<9.3	Deficient	0	1	0	0	0	0	0	0
(μM)	9.3–12	Marginal	6	0	1	1	0	0	1	1
	12–23	Normal	11	16	13	14	16	16	15	14
	>23	High	0	0	3	2	0	1	0	2
	$P=$			0.44		1.00		1.00		0.98
CP	<15	Deficient	0	1	0	1	0	0	0	0
(mg/dl)	>15	Adequate	17	16	17	16	16	17	16	17
	$P=$			1.00		1.00		0.86		0.86

duration) and these three all had liver copper concentrations exceeding 600 mg Cu/kg liver DM. The rest of the bolused sheep had bolus durations exceeding 240 days (~8 months) and maintained liver copper concentrations below this figure. This was a consequence of the previously mentioned variable dissolution problem, which has subsequently been addressed. However, copper supplementation should only be carried out in sheep with diagnosed low copper status, or with a history of copper deficiency

Table 6
Distribution of cobalt, selenium and copper status in trial 3

	Range	Status	Day 0		Day 21		Day 51		Day 105	
			Bolused	Control	Bolused	Control	Bolused	Control	Bolused	Control
Vitamin	<200	Deficient	1	2	0	0	0	0	0	0
B_{12}	200–400	Marginal	9	12	0	3	0	0	0	9
(pg/dl)	>400	Adequate	14	11	25	22	25	25	24	14
	$P=$			0.98		0.78		1.00		0.07
GSHPx	13.5	Deficient	0	0	0	0	0	0	0	0
(U/ml	13.5–28		5	6	3	7	0	3	0	2
PCV)	28–42		10	9	4	9	0	4	0	0
	>42	Adequate	10	10	18	9	25	18	25	22
	$P=$			1.00		0.59		0.42		0.98
PlCu	<9.3	Deficient	1	1	2	0	1	0	0	0
(μM)	9.3–12	Marginal	8	6	4	8	7	6	1	0
	12–23	Normal	16	18	18	17	17	19	24	23
	>23	High	0	0	1	0	0	0	0	1
	$P=$			1.00		0.82		1.00		0.98
CP	<15	Deficient	1	1	0	0	7	4	1	0
(mg/dl)	>15	Adequate	24	24	25	25	18	21	24	24
	$P=$			0.47		0.89		0.49		0.98

problems. Additional copper supplementation should not be carried out if copper-containing boluses are already present within the sheep.

The supplemental selenium increased the erythrocyte glutathione peroxidase activities in all three trials. Apart from day 21 in trial 3, when the bolused sheep showed a reduction compared to the controls in the numbers classified as marginal, the selenium status (GSHPx) of all bolused sheep could be classified as adequate subsequent to bolus administration. The control groups had some sheep with marginal status throughout trial 1, on days 42 and 63 in trial 2 and throughout trial 3. This indicates that the bolus was able to consistently prevent marginal selenium status from occurring in the bolused sheep. The reduced numbers of bolused sheep with marginal selenium status compared to the control sheep in trial 3 implies the ability of the bolus to correct a marginal status. Raised selenium status similar to that exhibited for the bolused sheep has been associated with increased immune responses in both sheep (Jelinek et al., 1988) and cattle (Nicholson et al., 1993).

The cobalt status (vitamin B_{12}) was adequate for all bolused sheep after bolus administration. However, there were control sheep in the marginal/deficient category for all trials and all days, except trial 3 day 51, indicating that the bolus was able to prevent marginal/deficient cobalt status from occurring in the bolused sheep. Further to this the reduction in the numbers of marginal/deficient bolused sheep from the day 0 distribution and comparison with the control sheep in trials 1 and 2 indicates a correction of marginal/deficient cobalt status.

The bolus has demonstrated a capacity to raise low selenium and cobalt status of the sheep to higher reference ranges. Sheep with health problems likely to be caused or exacerbated by low selenium and cobalt should benefit from receiving this type of bolus.

Although the selenium and cobalt status was significantly increased by the bolus, there were no significant effects on liveweight gain (Trials 1 and 2). The growth rates within these trials was low for both groups in both trials (Trial 1 bolused148 g/day, control 135 g/day: Trial 2 bolused 107 g/day, control 111 g/day) suggesting that selenium and cobalt status were not the limiting factors to growth. The most likely limiting factor to lamb growth was food intake, as the lambs were on trial, with no additional feed, during mid-summer with its associated reduction in grassland productivity.

5. Conclusions

The sintered soluble glass copper, cobalt and selenium bolus was able to prevent or correct deficient and/or marginal cobalt and selenium status of sheep throughout these trials. The bolus had little measured effect on the already adequate blood parameters of copper status, although the liver copper concentrations of the bolused sheep were higher in the trial for which they were analysed.

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