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The effect of a zinc, cobalt and selenium soluble glass bolus on the trace element status of extensively grazed sheep over winter

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Abstract

The effects of trace element deficiencies in lambs, particularly zinc, copper, cobalt and selenium, include decreased growth rates and increased mortality. However, trace element supplementation of sheep reared under extensive conditions has several logistical problems.

Two trials were designed to investigate the effect of a zinc, cobalt and selenium soluble glass bolus on the trace element status of out-wintered ewe lambs. In trial 1 600 8-month-old ewe lambs (500 Scottish Blackface and 100 North Country Cheviots) were allocated to two treatment groups; 300 were treated with a zinc, cobalt and selenium soluble glass bolus (zinc) and 300 were untreated (control). In trial 2, 315 8-month-old Scottish Blackface ewe lambs were allocated to three treatments: 105 were treated with the zinc, cobalt and selenium soluble glass bolus (zinc), 105 were treated with a copper, cobalt and selenium soluble glass bolus (copper) and the remaining 105 were untreated (control). Blood samples were collected immediately prior to giving boluses and again after approximately 4 months. These were assessed for zinc (plasma zinc concentration), cobalt (serum vitamin B_{12} concentration), selenium (erythrocyte glutathione peroxidase activity) and copper status (plasma copper concentration, caeruloplasmin, amine oxidase and superoxide dismutase activity and calculation of the ratio between the caeruloplasmin and plasma copper).

The zinc bolus in both trials significantly increased the plasma zinc concentrations ($P < 0{\cdot}001$ and $P < 0{\cdot}01$ respectively), erythrocyte glutathione peroxidase activities ($P < 0{\cdot}001$) and serum vitamin B_{12} concentrations ($P < 0{\cdot}001$). The copper bolus also significantly increased the erythrocyte glutathione peroxidase activities ($P < 0{\cdot}001$) and serum vitamin B_{12} concentrations ($P < 0{\cdot}001$) when compared with the controls but were not significantly different from the zinc group. The copper bolus significantly increased all of the copper status indicators ($P < 0{\cdot}01$) when compared with the control and zinc groups. However, in trial 1 when only the zinc and control groups were compared, the zinc bolus significantly increased the ratio ($P < 0{\cdot}001$) and serum caeruloplasmin ($P < 0{\cdot}001$) and erythrocyte superoxide dismutase ($P < 0{\cdot}01$) activities. These responses were not observed in trial 2 with the erythrocyte superoxide dismutase being significantly reduced in the zinc group when compared with the control group ($P < 0{\cdot}001$).

The zinc, cobalt and selenium soluble glass bolus increased the status of all three trace elements consistently for a period of at least 100 days. The increases of cobalt and selenium status were similar to those achieved using the copper, cobalt and selenium bolus, which also increased the copper status of the sheep.

Keywords: *controlled release, sheep, supplements, trace elements.*

Introduction

Trace element deficiencies in ruminants, such as zinc, copper, cobalt and selenium, have profound effects on health and production leading to poor growth rates and increased mortality (Underwood, 1981). These deficiencies occur most frequently in the grazing animal where the use of extra food is limited. Supplementation of these grazing ruminants with trace elements can prove difficult. For example, the free-access methods (minerals, licks and blocks) available require the animal to sense any deficiency and seek out suitable supplements. The sensing of requirements (expression of pica behaviours) has been seen in humans and animals, although only in cases of extreme deficiency (Chen *et al.*, 1985; Hambridge *et al.*, 1986; Kendall and Telfer, 2000). Free access systems also suffer from variable intakes (Kendall, 1977). Oral drenches require frequent dosing in order to prevent deficiencies due to the short acting response of this type of supplement (Kendall *et al.*, 2000). Due to the extensive nature of hill sheep production, pasture dressing and water supplementation are difficult to apply in practice. Injections require long term storage within the animal or frequent dosing. Supplemental feeding is difficult due to the extensive conditions and will add significantly to the cost of production.

Controlled release intra-ruminal strategies, such as boluses or needles, provide relatively long-acting trace element supplementation. This form of supplementation does not require the animal to consistently consume supplementary food, blocks, or free-access minerals and neither is the animal required to store the elements supplemented for later use.

Previous work has shown that a sintered copper, cobalt and selenium soluble glass intra-ruminal bolus method of supplementation can release these elements in biologically available forms to sheep and cattle (Kendall *et al.*, 1999, 2000 and 2001; Moeini *et al.*, 1997). A sintered zinc, cobalt and selenium soluble glass intra-ruminal bolus has been developed to provide cobalt and selenium supplementation in situations where additional copper could prove detrimental, such as when sheep are housed, given additional supplements or are from breeds of low copper tolerance (e.g. North Ronaldsay, Texel, Blue Faced Leicester). However, this type of bolus may have benefits when used in a different situation from that of the original design specification. The zinc content of this bolus could prove beneficial for udder health (reduced mastitis and increased rekeratinization) (Martin *et al.*, 1996; Whitaker *et al.*, 1996), reduce incidence of footrot (Demertzis *et al.*, 1978), increase immune response (Gershwin *et al.*, 1987), increase wound healing (Wacker, 1976) and conceivably have a rôle in the prevention of broken mouth (Miller, 1991). However, the zinc could prove to be detrimental to the copper status, as a zinc-copper interaction is well documented (O'Dell, 1989), therefore copper status was measured in both trials.

The trials reported were designed to study the effects of the zinc, cobalt and selenium soluble glass bolus on the zinc, cobalt, selenium and copper status of ewe lambs of copper tolerant breeds, extensively grazed over winter and therefore not at risk of copper toxicity. A copper, cobalt and selenium soluble glass bolus was used in trial 2 to enable comparison of selenium and cobalt supplementation with that from the zinc containing bolus.

Material and methods

Trial 1

Six commercial sheep farms in the Tayside region of Scotland each provided 100 8-month-old ewe lambs (five flocks of Scottish Blackface and one flock of North Country Cheviots) that were to be over-wintered without producing a lamb crop. Each flock was split into two groups of 50 lambs with one group having a 33 g zinc bolus administered and the other group receiving no bolus (Table 1). The sheep were grazed throughout the trial with no concentrates or mineral supplements offered.

Trial 2

Three different commercial sheep farms in the Tayside region of Scotland each provided 105 8-month-old Scottish Blackface ewe lambs that were to be over-wintered without producing a lamb crop. Each flock was split into three groups of 35 with one group given a 33 g zinc bolus, the second group received a 33 g copper bolus and the third group received no bolus (Table 1). The sheep were grazed throughout the trial with no concentrates or mineral supplements offered.

Analysis

Heparinized and unheparinized blood samples were collected *via* jugular venipuncture into Monovette tubes (Sarstedt) from all lambs immediately prior to treatment (December) (day 0) and a further sample was taken after approximately 4 months (March/April). The heparinized blood samples were analysed for haemoglobin concentrations (Hb), haematocrit values (Hc) and aliquots were stored at -20°C for subsequent analysis of erythrocyte glutathione peroxidase activities (selenium status) and erythrocyte superoxide dismutase activities (copper status). The plasma was removed after centrifugation, diluted 1 in 5 with 0·1 mol/l HCl (Analar, BDH) and stored at –20°C prior to

Table 1 *The manufacturer and elemental composition of bolus treatments administered*

Treatment	Bolus composition (% w/w)				Trade name
	Zinc	Cobalt	Selenium	Copper	
Zinc	15·2	0·5	0·15		Zincosel®, Telsol Ltd
Copper		0·5	0·15	13·2	Cosecure®, Telsol Ltd
Control	No bolus administered				N/A

subsequent analysis of zinc and copper concentrations. The unheparinized samples were centrifuged to provide serum and aliquots were stored at –20°C for subsequent analysis of caeruloplasmin and amine oxidase activities (copper status) and vitamin B_{12} concentrations (cobalt status).

Haemoglobin concentrations were determined using Hemocues (Ängelholm, Sweden). Plasma zinc (PlZn) and copper (PlCu) concentrations were determined by atomic absorption spectrophotometry at 213·9 nm with background correction and at 324·8 nm respectively on a Pye SP9 AA spectrophotometer. Enzymes were analysed using the following methods, adapted for the Cobas Mira autoanalyser (Roche): erythrocyte glutathione peroxidase (GSHPx) activities (Paglia and Valentine, 1967), serum caeruloplasmin (CP) concentrations (Henry *et al.*, 1974), erythrocyte superoxide dismutase (SOD) activities (Misra and Fridovich, 1977), serum amine oxidase (AMOX) activities (Mulryan and Mason, 1992). Serum vitamin B_{12} concentrations were determined by boiling radioassay (ICN). The ratio between CP and PlCu was calculated and used as a further indicator of copper status (Mackenzie *et al.*, 1997).

Statistical analysis
The results were pooled for each trial and analysed by ANOVA using day 0 as a covariate and farm as a cofactor using GLM on MINITAB 11. For trial 2 least significant differences were used to determine statistical significance between the individual group means.

Results

In trial 1, there was no significant effect of treatment on lamb live weight (Table 2). The sheep given a zinc bolus had significantly higher ($P < 0·001$) plasma zinc concentrations, erythrocyte glutathione peroxidase activities and serum vitamin B_{12} concentrations (Figure 1a, b and c, respectively). Compared with the control group, the zinc group had an increased copper status (Table 2) with significant increases in superoxide dismutase ($P < 0·01$), caeruloplasmin and the CP/PlCu ratio ($P < 0·001$), although plasma copper concentrations and amine oxidase activity

Table 2 *The effect of the zinc bolus on live weight, copper status (PlCu, CP, CP:PlCu ratio, AMOX, SOD) and haematological parameters (Hb, Hc) for trial 1 (pre-treatment values (day 0) are included for reference)*

Parameter†	Unit	day 0		Control		Zinc		Significance‡
		Mean	s.e.	Mean	s.e.	Mean	s.e.	
Weight	(kg)	38·4	0·30	41·6	0·19	41·8	0·19	
PlCu	(μmol/l)	11·7	0·16	8·4	0·16	8·5	0·16	
CP	(mg/dl)	24·8	0·51	15·2	0·40	17·5	0·39	***
CP:PlCu		2·10	0·032	1·80	0·030	2·11	0·031	***
AMOX	(U/ml)	79·9	1·32	59·6	1·28	61·2	1·29	
SOD	(U/g Hb)	2642	63·6	2664	45·4	2867	45·4	**
Hb	(g/l)	142·0	0·57	133·7	0·48	131·7	0·48	**
Hc	(%PCV)	42·0	0·20	42·0	0·21	40·3	0·21	***

† PlCu = plasma copper; CP = serum caeruloplasmin; AMOX = serum amine oxidase; SOD = erythrocyte superoxide dismutase; Hb = haemoglobin; Hc = haematocrit; PCV = packed cell volume.
‡ Levels of significance are between the zinc and control groups at the second sampling.

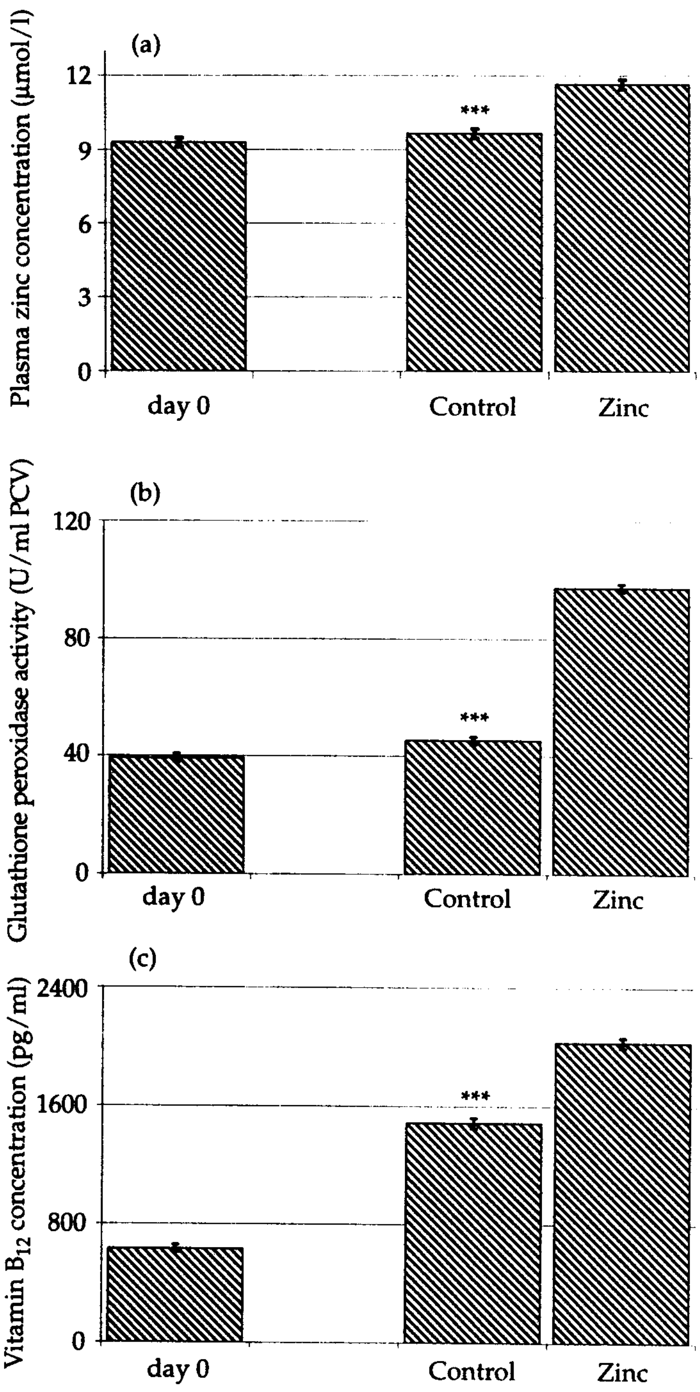

Figure 1 Mean values (± s.e.) for (a) plasma zinc concentration, (b) glutathione peroxidase activity and (c) vitamin B_{12} concentration of the control and zinc groups with the day 0 covariate value for trial 1.

were not significantly raised. Haemoglobin concentration ($P < 0.01$) and haematocrit ($P < 0.001$) were significantly higher for the control group when compared with the zinc group.

In trial 2, there were no significant differences in live weight between the zinc and control, and copper and control groups, however, the copper group were significantly heavier than the zinc group ($P < 0.05$) (Table 3). The zinc bolus significantly increased plasma zinc concentration ($P < 0.01$) when compared with the copper and control groups, which were not significantly different (Figure 2a). Glutathione peroxidase activity (Figure 2b) and vitamin B_{12} concentrations (Figure 2c) were significantly higher for both the zinc and copper bolus groups ($P < 0.001$) with no significant difference between the two bolus types. The copper group had significantly increased plasma copper concentration, caeruloplasmin activity and amine oxidase activity ($P < 0.001$) compared with the other two groups which did not differ significantly (Table 3). The superoxide dismutase activity of the copper group was significantly higher than that of the control group ($P < 0.01$) which was in turn significantly higher than the zinc group ($P < 0.001$). Haemoglobin concentration was significantly higher for the copper group than the control ($P < 0.01$) and the zinc group ($P < 0.05$). The haematocrit was higher for the control group ($P < 0.05$) than either bolus group, which did not differ significantly.

Discussion

The sheep that received a soluble glass bolus of either form (zinc or copper) had both significantly increased selenium (GSHPx) and cobalt (vitamin B_{12}) status at the second blood sampling (100 to 133 days after treatment). Apart from one farm in each trial all the flocks had a low selenium status as indicated by individual farm control group means. However, the cobalt status of all of the flocks was adequate on every farm as indicated by the individual farm control group means. The copper bolus sheep also had a significantly higher copper status (CP, PlCu, ratio, SOD, AMOX), whilst the zinc bolus sheep exhibited significant increases in zinc status (PlZn). The zinc bolus also exerted a significant effect on the enzymes used to assess copper status in trial 1, when CP (and consequently the CP/PlCu ratio) and SOD were significantly higher for the zinc group ($P < 0.001$, $P < 0.001$ and $P < 0.01$ respectively). However, in trial 2 the converse was found with the zinc bolus significantly lowering the SOD activity ($P < 0.001$). The exact mechanism for the zinc bolus group exhibiting increased enzyme parameters of copper status in trial 1 is not understood. However, it could be due to the zinc, cobalt or selenium component of the bolus or a combination of these elements. If zinc alone was supplemented a depression in copper status might be expected due to the well-documented interaction between these two elements (O'Dell, 1989). However, the levels of zinc generally required to depress copper status (Bremner *et al.*, 1976) are much higher than those released from the bolus.

Table 3 *The effects of the zinc and copper boluses on live weight, copper status (PlCu, CP, CP:PlCu ratio, AMOX, SOD) and haematological parameters (Hb, Hc) for trial 2 (pre-treatment values (day 0) are included for reference)*

		day 0		Control		Zinc		Copper	
Parameter†	Unit	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.
Weight	(kg)	34·8	0·30	33·2[ab]	0·33	33·0[a]	0·33	34·1[b]	0·35
PlCu	(μmol/l)	12·1	0·15	9·0[a]	0·23	8·8[a]	0·24	11·8[b]	0·24
CP	(mg/dl)	27·4	0·60	16·3[a]	0·60	17·3[a]	0·61	26·4[b]	0·64
CP:PlCu		2·25	0·033	1·81[a]	0·038	1·90[a]	0·039	2·24[b]	0·041
AMOX	(U/ml)	78·9	1·31	51·7[a]	1·72	49·7[a]	1·77	76·1[b]	1·83
SOD	(U/g Hb)	2426	56·6	3023[a]	68·3	2658[a]	70·7	3299[c]	73·0
Hb	(g/l)	144·4	0·57	119·4[a]	0·93	119·6[a]	0·96	123·1[b]	0·98
Hc	(%PCV)	42·4	0·23	40·5[a]	0·36	39·1[b]	0·38	39·3[b]	0·38

[a,b,c] Different superscripts indicate a significant difference ($P < 0{\cdot}05$) between groups at the second sampling.
† PlCu = plasma copper; CP = serum caeruloplasmin; AMOX = serum amine oxidase; SOD = erythrocyte superoxide dismutase; Hb = haemoglobin; Hc = haematocrit; PCV = packed cell volume.

Previous balance studies have shown zinc from the zinc bolus to be biologically available to sheep (Kendall and Telfer, 2000). Cobalt and selenium from the zinc bolus have also been shown to be biologically available to lambs in lowland summer grazing situations (Kendall *et al.*, 1997). Recovery of zinc boluses from previous trials has shown that they last for over 100 days (Kendall *et al.*, 1997). The release period of the soluble glass zinc boluses (>100 days) means that supplemental cobalt and selenium with zinc (or copper from the copper bolus) can be supplied to sheep all year round with a maximum of three handling sessions required. The boluses negate the need to supply these trace elements in free-access minerals, which can have variable intakes from zero to excessive (McDowell, 1992), or the supply of supplemental food when only mineral supplementation is required. Food blocks, which have been shown to be useful for supplementation in extensive situations, also have the problems of variable intakes with up to 50% of ewes choosing not to consume any of the supplement (Kendall, 1977). Oral supplementation requires frequent regular dosing in order to maintain an effective response, a yeast chelate drench and inorganic drench were shown to be effective for the correction of clinical copper deficiency in sheep for only approximately 2 weeks (Kendall *et al.*, 2000). Supplementation of drinking water also suffers from variable intakes, often related to the weather (Towers *et al.*, 1976). The use of commercially managed hill sheep flocks in this trial demonstrates the usefulness of the boluses in this situation, where sheep are often left with no managerial contact for months between gatherings for husbandry practices such as shearing, dipping or drafting of lambs.

The zinc, cobalt and selenium soluble glass bolus has previously been demonstrated to give increased humoral immune response in grazing lambs (Kendall *et al.*, 1997). The increases in one or more of the trace elements, as obtained in these trials, are likely to give responses in extra immunocompetence, especially if any marginal deficiencies are prevented. Further supplementation of selenium to apparently normal status animals has resulted in an increased immune response in cattle, sheep and other experimental animals (Nicholson *et al.*, 1993; Jelinek *et al.*, 1988; Spallholz, 1981). Cobalt deficiency has been shown to give a reduced immune response in sheep with a decreased lymphoblastic response to *Mycobacterium paratuberculosis* challenge (Vellema *et al.*, 1996). Copper deficiency has been shown to give reduced killing capacity of leukocytes in both sheep and cattle (Jones and Suttle, 1981). Zinc is extensively involved in immune function and even marginal deficiency will affect immune response (Gershwin *et al.*, 1987). Oral supplementation of zinc to normal status human subjects resulted in increased lymphocyte proliferative responses (Reddy and Frey, 1990). Marginal trace element status, as seen in many of the individual farm control group means, implies that the bolus may have a rôle in the maintenance/promotion of immunocompetence in extensively grazed hill sheep.

In conclusion, the zinc, cobalt and selenium soluble glass bolus increased the status of all three trace elements consistently for a period of at least 100 days. The increases of cobalt and selenium status were similar to those achieved using the copper, cobalt and selenium soluble glass bolus, which also increased the copper status of the sheep. The zinc

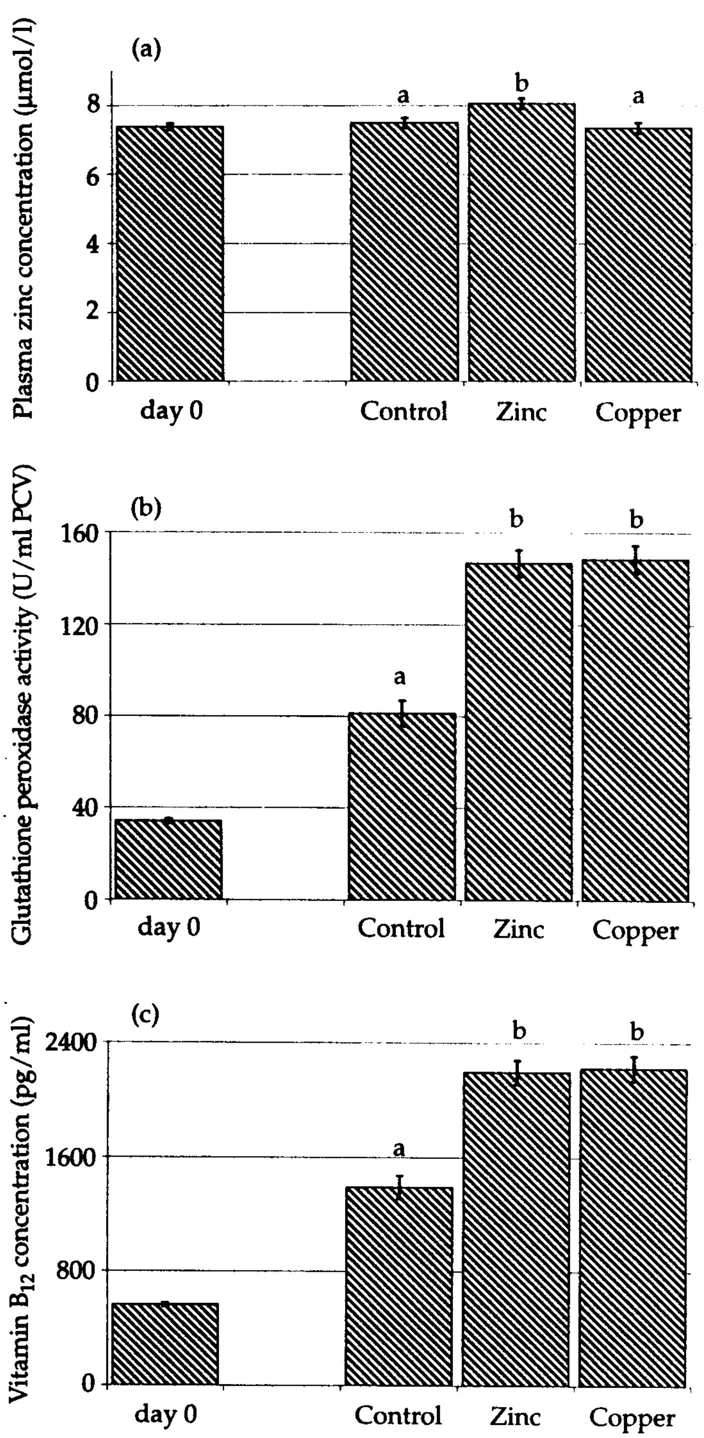

Figure 2 Mean values (± s.e.) for (a) plasma zinc concentration, (b) glutathione peroxidase activity and (c) vitamin B_{12} concentration of the control, zinc and copper groups with the day 0 covariate value for trial 2.

bolus proved to be an appropriate method of increasing the selenium and cobalt status of extensively grazed over-wintered sheep, with the additional benefit of increasing zinc status.

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