To refine and confirm the level of selenium and iodine supplementation for breeding ewes

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EBLEX

Prepared by:
ADAS UK Ltd
Pendeford House
Pendeford Business Park
Wolverhampton
WV9 5AP

PRINCIPAL WORKERS

K. A. Phillips
K. P. A. Wheeler
H. Fuller

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Executive summary

Current recommended dietary allowances for selenium and iodine are based on the 1983 ARC recommendations. For selenium the recommended dietary allowance in sheep is 0.1 mg/kgDM but anecdotal evidence suggests that higher levels could be beneficial in terms of fertility, lamb viability and immunity. Selenium acts with vitamin E to protect tissues against oxidation and the breakdown of cell membranes and is also important for immune function. Lack of selenium is most widely recognised as white muscle disease (WMD), ill-thrift, and infertility. Diagnosis of deficiency is usually by blood sampling and measuring levels of the enzyme, glutathione peroxidase (GSH-Px), which contains selenium. However there is debate within the industry as to the desirable blood reference range for GSH-Px.

The recommended dietary allowance for iodine in sheep is 0.5 mg/kgDM in winter, 0.15 mg/kgDM in summer and 2.0 mg/kgDM in the presence of goitrogens (ARC, 1983). Iodine is a component of the hormone thyroxine which controls energy metabolism and is also essential for foetal growth and development. Iodine deficiency is typically associated with an enlarged thyroid gland, commonly known as a goitre. Typical signs are late abortions presenting still-born or weak lambs. Weighing the thyroid and taking samples from the thyroid gland of stillborn lambs is the best way of diagnosing iodine deficiency. In adult animals, blood can be tested for the hormone thyroxine (T4). While this test is readily available, ewe thyroid hormone concentrations before mating and during mid-pregnancy have been reported to be poor indices of deficiency. A more recent test is plasma inorganic iodine (PII) but whilst reference ranges have been established for cattle, there are limited data for sheep on typical levels for PII.

The objective of the study reported here was to refine and confirm the level of selenium and iodine supplementation for breeding ewes by evaluating the effect of differing levels of selenium and iodine supplementation on performance.

For this study four farms were selected on the basis of historic trace element problems. Two had a history of selenium deficiency (Hereford and Shropshire). The farms in Cornwall and Northumberland were selected by the farm vets as having clinical signs of iodine deficiency. On the Northumberland farm, goitre and thyroid hyperplasia had been seen in stillborn lambs, whilst on the Cornish farm, neonatal goitre had been observed in stillborn calves.

The trial used a fully randomised design with 30 to 50 ewes per treatment group. At approximately three weeks pre-tupping ewes were weighed and condition scored and randomly allocated to one of three treatment groups as follows:

Selenium study

1. Base line selenium plus 0.1 mg Se/kg DM
2. Base line selenium plus 0.2 mg Se/kg DM
3. Base line selenium plus 0.3 mg Se/kg DM

Treatment boluses also supplied a standard dose of 2.0 mg iodine/kg DM.

Iodine

1. Baseline iodine plus 0.5 mg I/kg DM
2. Baseline iodine plus 1.0 mg I/kg DM
3. Baseline iodine plus 2.0 mg I/kg DM

Treatment boluses also supplied a standard dose of 0.2 mg selenium/kg DM.

Blood samples were taken for trace element analysis prior to the start of the study to establish baseline levels (GSH-Px, vitamin B_{12}, plasma copper and plasma inorganic iodine/thyroxine). Further blood sampling was planned for pregnancy scanning and late pregnancy with an additional blood sampling in November for the iodine study. On each

farm samples of grass and conserved forage (where offered) were taken and analysed for selenium, iodine, cobalt, copper, zinc and manganese and the copper antagonists, molybdenum, sulphur and iron. Sheep were managed in mixed treatment groups throughout pregnancy. Ewes were weighed and condition scored at the start of the study, at scanning and/or pre-lambing and once post-lambing. Lamb live weights and daily gain were assessed to weaning.

Samples of grass and conserved forage were collected at the start of the study from all four farms. For the farms with a history of low selenium, the sampling confirmed that selenium concentrations in the forage were below the recommended levels for ruminants. However, iodine level in the forage from the other farms were within the normal range.

On the selenium farms baseline blood sampling of ewes confirmed low or marginal GSH-Px concentrations at the start of the study. Selenium supplementation at 0.1, 0.2 and 0.3 mg per kg DM all significantly increased the GSH-Px levels on both farms with the treatment means across the two flocks all above 140 U/ml RBC at 120-136 days post bolus. Ewe weights and body condition during pregnancy were unaffected by the level of selenium supplementation. On the Herefordshire farm, no difference in ewe productivity was observed between the three treatment groups. Data on the productivity of ewes on the Shropshire farm is not available as the farmer withdrew from the project due to ill health and terrible weather.

On the iodine farms, PII concentrations were low at the start of the study, and increased after bolus administration in both cases. In Northumberland the mean PII concentration at the start was 40 ng/ml and bolus supplementation increased the PII concentration in proportion to increasing levels of iodine in the bolus. At day 42 after bolus administration mean PII concentrations of bolused ewes were all above 105 ng/ml, the level considered normal by the AHVLA for iodine supplemented animals. By day 91 only the ewes in the 2.0 mg group had a mean PII level of >105ng/ml.
On the Cornish farm, the PII concentration of a pooled sample at the start of the study was low at 14 ng/ml. Following bolus administration, the mean PII concentration in supplemented ewes did not reach the 105 ng/ml threshold at either sampling point (days 59 and 119 post bolus). At day 59 post bolus, mean PII levels for the three treatment groups (0.5, 1.0 and 2.0 mg I) were 25.8, 61.8 and 66.7 ng/ml respectively. At day 119 post bolus, the mean PII levels for the three groups were all low (less than 20 ng/ml in all treatment groups).
As in the selenium study, ewe weights and body condition during pregnancy were unaffected by the level of iodine supplementation pre-tupping; differences in lamb vigour and lamb losses between treatments were not observed on either of the trial farms and no clinical signs suggestive of iodine deficiency were seen. Lamb performance was unaffected by the treatment regime of the ewes. There was a tendency for lambs on the low iodine (0.5 mg I) treatment to be lighter at weaning on the Cornish farm, but the difference was not statistically significant**.**

Conclusions

The primary objective of this study was to refine and confirm the level of selenium and iodine supplementation for breeding ewes by evaluating the effect of differing levels of selenium and iodine supplementation provided by intraruminal bolus on performance. Results need to be treated with caution since the data is limited to only one farm for selenium and two for iodine.
However, in this study pre-tupping administration of a sustained release intra-ruminal bolus providing 0.1 mg selenium per kg DM was found to provide adequate levels of

selenium on a known selenium deficient farm, with no observed benefits in supplementing with 0.2 or 0.3 mg selenium per kg DM.

For iodine, the results have raised more questions than answers. The duration of supplementation from the boluses, as measured by PII concentrations was less than expected on both farms. Also, PII concentrations did not rise as high as expected on one farm. Questions have been raised as to whether the current guidelines for the interpretation of PII are suitable for assessing the adequacy of iodine intake in pregnant ewes.

Background

Selenium

The current recommended dietary allowance for selenium in sheep is 0.1 mg/kgDM (ARC, 1983) but anecdotal evidence suggests that higher levels could be beneficial in terms of fertility, lamb viability and immunity. NRC recommendations (2006) are significantly higher and advise a level of 0.3 mg/kgDM or as set out below:

Maintenance	0.00025 mg/kg BW/AC
Growth	0.50 mg/kg LWG/AC
Pregnancy (last one-third)	0.0025 mg/kg LBW/AC
Lactation	0.14 mg/kg MY/AC

where: BW = body weight, LBW = litter birth weight, MY = milk yield, kg/day and AC = absorption coefficient (forages = 0.31, concentrates = 0.60)

Suttle (2005) suggests that 0.025 to 0.05 mg/kgDM would be an indicator of a marginal risk of selenium deficiency.

Selenium acts with vitamin E to protect tissues against oxidation and the breakdown of cell membranes and is also important for immune function. The selenium requirements of stock are related to the vitamin E content of the diet. For diets low in vitamin E the requirements for selenium are increased and vice versa.

Lack of selenium is mostly widely recognised as white muscle disease (WMD), ill-thrift, and infertility. A deficiency in young lambs can show as an inability to stand because their leg muscles are affected. In young growing animals, selenium deficiency may be a cause of ill thrift. Lack of selenium can also cause poor reproductive performance. In females, it can cause early embryonic death, resulting in poor scanning figures in sheep but inadequate selenium also affects male fertility, so where a deficiency is identified, it is important that rams are also supplemented.

Excess selenium is toxic to sheep and cattle, although the risk is very much less than with copper, and cases of selenium toxicity are rare.

Diagnosis of deficiency is usually by blood sampling and measuring levels of the enzyme, glutathione peroxidase (GSH-Px), which contains selenium. However there is debate within the industry as to the desirable blood reference range for GSH-Px (anecdotally higher levels are being recommended to confer improved immunity to disease) and this work would help to confirm the correct level for sheep.

Iodine

The current recommended dietary allowance for iodine in sheep is 0.5 mg/kgDM in winter, 0.15 mg/kgDM in summer and 2.0 mg/kgDM in the presence of goitrogens (ARC, 1983).

The NRC (2006) recommendations are 0.5 mg/kg diet DM for growing and mature, non-lactating sheep and 0.8 mg/kg diet DM for lactating ewes.

Iodine is a component of the important hormone thyroxine which controls energy metabolism. It is also essential for foetal growth and development. It is needed for the thyroid, a gland in the throat to function properly. Iodine deficiency is typically associated with an enlarged thyroid, commonly known as goitre. Typical signs are late abortions

presenting still-born or weak lambs. Neonatal mortality is markedly increased (Sargison et al, 1998).

Pregnant and lactating animals have a much higher iodine requirement compared to 'dry' stock, and pasture is often unable to fully satisfy requirements on its own. Some forage crops such as brassicas contain substances called goitrogens, which interfere with thyroxine production. Animals grazing brassica crops have an increased requirement for iodine compared with those grazing grass.

Taking samples from the thyroid gland from stillborn lambs is the best way of diagnosing iodine deficiency. In adult animals, blood can be tested for the hormone thyroxine (T4). While this test is readily available, ewe thyroid hormone concentrations before mating and during mid-pregnancy have been reported to be poor indices of deficiency (Sargison et al 1998; Clark et al 1998). A more recent test is plasma inorganic iodine. While reference ranges have been established for cattle, there are limited data for sheep on what are typical levels for plasma inorganic iodine. Aumont et al (1989) monitored the plasma inorganic iodine (PII) level of ewes receiving diets with differing iodine content. The PII of ewes never went above 20 µg/l when the iodine content of the ration was between 0.26 – 0.36 mg/kg DM.

This PII level would be considered deficient in cattle. When fed iodine at 2.0 mg/kg DM – the maximum recommended by the ARC – the iodine peaked at 80 µg/l. This is below the threshold considered normal in cattle (101 – 300 µg/l). This work would provide much needed confirmation of the range and typical status of breeding ewes.

Selenium and iodine work together in thyroid metabolism and have a key role in mobilisation of brown adipose tissue in newborn lambs. They therefore have a role in improving lamb survival.

Interactions between selenium and iodine

Both iodine and selenium are required for optimal thyroid function. Iodine is required as a constituent of thyroid hormones, whilst selenium is required to form the enzymes (deiodinases) that convert thyroxine (T4) to its physiologically active form (T3). A deficiency of selenium can compound the harmful effects of iodine deficiency, whilst supplementing with selenium can ameliorate the effects of iodine deficiency.

In this project, the aim is to assess responses to the two trace elements independently. On the iodine study farms, the aim will be to maintain the selenium status in the trial ewes well above the reference range, by monitoring GSH-Px levels. In the selenium study group, ensuring that iodine levels are satisfactory is slightly more difficult as iodine status is harder to assess from blood samples. On all farms, monitoring the ratio of thyroid weight (in grams) to lamb birthweight (in kg) of stillborn lambs will provide additional information on iodine status in the trial ewes.

Interactions with other trace elements

On the selected farms, either selenium or iodine deficiency have been previously identified in sheep. The aim will be to ensure that the copper and cobalt status of all the ewes in the studies remain well within the standard reference ranges throughout.

Objective

To refine and confirm the level of selenium and iodine supplementation for breeding ewes by evaluating the effect of differing levels of selenium and iodine supplementation on performance.

Materials and Methods

Trial sites

A total of four farms were recruited, two each for the iodine and selenium studies. The farms selected had previously been identified as having selenium or iodine deficiencies. The selenium study farms were located on commercial farms in Shropshire and Herefordshire and iodine farms in Cornwall and Northumberland. The following provides an overview of the sheep flocks and their trace element history on each of the farms.

Shropshire – Selenium. An upland farm near Bishops Castle in Shropshire with much of the land classified as LFA. The ewe flock predominantly consists of Lleyn ewes that are tupped on grass before overwintering on stubble turnips and lambing outside from the third week of March. Selenium deficiency is not uncommon in this area and blood sampling in 2009 -2010 confirmed low GSH-Px levels in ewes and lambs in the autumn when stock had been reliant on grass alone for the summer. Ewes now receive a multi-trace element bolus pre-tupping.

Herefordshire – Selenium. A lowland organic farm in north Herefordshire, with a mix of temporary and permanent pasture. The 400 ewe flock predominantly consists of Romney and Lleyn ewes that are run on a grass based system lambing outside in April. Selenium deficiency was confirmed several years ago through blood sampling. Supplementation of breeding ewes is carried out by administering a multi-trace element bolus. The study ewes had last received a bolus in March 2012 which would have provided continual supplementation over the summer grazing period.

Northumberland – Iodine. A hill farm running a total of 1000 Blackface ewes in hefted flocks across three hills. Iodine deficiency was suspected around three years ago when a number of stillborn lambs from one group were found to have hugely enlarged thyroid glands (up to 104g in one case). Thyroid hyperplasia was diagnosed through histopathology. The following season ewes from this group were supplemented with a multi-trace element bolus and no problems were seen in the resulting lamb crop. However, similar symptoms of enlarged thyroids were seen in stillborn lambs from a different group of ewes. The farm joined the study in autumn 2012 to investigate the problem further. Ewes that were put onto the trial had previously been in groups affected by clinical symptoms in a previous year.

Cornwall – Iodine. A lowland farm near Launceston extending to around 300 acres in total, of which 250 acres is grassland (around 80% grass is permanent pasture). The farm currently carries a flock of 900 north country mule ewes and until recently has also supported a suckler herd. Clinical symptoms of iodine deficiency were originally diagnosed in the suckler herd several years ago on the basis of stillborn calves with enlarged thyroids and are not uncommon in the surrounding area. As a result the sheep flock has received iodine supplementation through mineral supplementation and an oral drench of potassium iodide in late pregnancy.

Blood samples

Prior to the start of the study blood samples were taken from 8 ewes of representative ages on each farm for trace element analysis (GSH-Px, vitamin B_{12}, plasma copper and plasma inorganic iodine/thyroxine) to establish baseline levels.

Subsequently the plan was to blood sample ewes from each treatment at scanning (Jan/Feb 2013) and four weeks before lambing (with an additional sampling for iodine farms in November 2012). However on three of the farms the scanning assessment was significantly delayed resulting in the pre-lambing blood sampling being abandoned.

For selenium farms subsequent blood samples were analysed for GSH-Px whilst samples from iodine farms were analysed for PII and/or thyroxine (T4).

Grass and conserved forage samples

On each farm grass/forage samples were taken in October from four fields that were to be grazed by ewes in pregnancy. Samples were taken from representative areas with care taken to minimise contamination of the sample by soil. A pooled sample was sent to NRM laboratories for analysis of the trace elements selenium, iodine, cobalt, copper, zinc and manganese and the copper antagonists, molybdenum, sulphur and iron. With the exception of selenium, trace elements were assessed using ICPMS (Inductively coupled plasma mass spectrometry). Selenium levels can be unreliable particularly at lower levels using this method so were analysed by hydride atomic fluorescence spectroscopy (AFS). In a previous study looking at trace element status in cattle and sheep the laboratory highlighted that the ICPMS method which is typically used in the wider industry can not be considered to be reliable for iodine, although it does give a broad indication of iodine content.

Where supplementary forage was offered to ewes, samples typical of that fed in late pregnancy were taken for trace element analysis in Jan/Feb 2013. Two or three samples were taken from each farm and sent for analysis as above.

Experimental design and treatments

The trial used a fully randomised design with 30-50 ewes per treatment group. Where appropriate yearling ewes were allocated to treatment separately to older ewes.

At approximately three weeks pre-tupping ewes were weighed and condition scored and randomly allocated to one of three treatment groups as follows:

Selenium study

1. Base line selenium plus 0.1 mg Se/kg DM
2. Base line selenium plus 0.2 mg Se/kg DM
3. Base line selenium plus 0.3 mg Se/kg DM

Treatment boluses also supplied a standard dose of 2.0 mg/kg DM of iodine

Iodine

1. Baseline iodine plus 0.5 mg I/kg DM
2. Baseline iodine plus 1.0 mg I/kg DM
3. Baseline iodine plus 2.0 mg I/kg DM

Treatment boluses also supplied a standard dose of 0.2 mg/kg DM of Se

On each study the additional selenium or iodine was provided in rumen boluses manufactured by Agrimin Ltd. The trial boluses were based on commercially available products with either the Se or I content varied for the study. Boluses were administered either by the farmer or by Agrimin staff as ewes were randomised into their treatment groups and were assumed to provide continual supplementation from approximately 3 weeks pre-tupping through to lambing.

A record of any additional dietary supplementation including trace elements was noted throughout pregnancy. Trace element profiles for ewes during pregnancy were plotted using the levels reported in the forage and the declared analysis of other feeds and supplements. The daily amount of Se and I released from the trial boluses was assumed to be constant throughout but in reality is likely to vary, being higher in the first 6-8 weeks and then falling slowly.

Animal assessments

Originally ewes were to be weighed and condition scored at randomisation, pregnancy scanning and four weeks pre-lambing to coincide with blood sampling. However, due to scanning assessments being later than planned on some farms only two of the farms (iodine farms) completed all pre-lambing assessments. Post-lambing, ewes were weighed and condition scored once to coincide with lamb weighing at 5-8 weeks with the exception of one of the iodine farms where this assessment was delayed until weaning.

Lambing assessments were carried out by the host farmers. The original plan was for farmers to record lamb birth weights and lamb vigour (using a simple scale of time to stand and suckle). In addition they were asked to weigh any still born lambs or those dying in the first 24 hours and submit them for an assessment of the thyroid gland. In practice detailed record keeping and monitoring of individual litters was not possible on the farms. The poor weather conditions in late March 2013 forced one of the selenium farms to withdraw from the study. On all of the remaining farms identification of lambs to treatment groups was achieved and an assessment of average lamb birth weights was made along with any evidence of differences in lamb vigour between treatment groups.

Lambs were weighed at least twice on all farms, once at 5-8 weeks of age and again at weaning (or sale if earlier).

Statistical analysis

Animal performance data and blood trace element data were analysed using ANOVA (Genstat 8th edition).
The chi square test was used to analyse categorical data including ewe body condition score and pregnancy scanning data.

Results

Grass and forage analysis

Analysis of a pooled sample from four grazing fields revealed selenium levels below recommendations on the Herefordshire and Shropshire farms whilst levels on the iodine farms were above requirements (Table 1). Iodine levels on the Cornwall farm met requirements for growing and dry stock but were below recommendations for pregnant and lactating stock. The Northumberland farm had the highest levels of iodine in the grazing samples being above that required for pregnant and lactating stock. The Shropshire and Herefordshire grass samples reported the lowest iodine levels with neither exceeding the recommended levels for dry and growing stock and therefore below recommendations for pregnant and lactating ewes. Levels of cobalt, zinc and manganese were at or above recommended levels on all farms. Copper levels were marginally below recommended levels on the Northumberland farm but above recommendations on the other three farms. Levels of the copper antagonists molybdenum and sulphur were higher than recommended on the farms (with the exception of S in Northumberland) which could impact on copper availability.

Table 1. Trace element levels in grazing fields and recommended levels (reported on a dry matter basis)

	Selenium farms		Iodine farms		Recommended levels to prevent deficiency
	Herefordshire	Shropshire	Cornwall	Northumberland	
					More than
Selenium (mg/kg)	0.03	<0.02	0.15	0.38	0.05
Iodine (mg/kg)	0.18	0.20	0.31	0.65	0.2*/0.5**
Cobalt (mg/kg)	0.21	0.11	0.16	0.11	0.11
Copper (mg/kg)	8.1	10.5	10.7	4.5	5 (sheep)
Zinc (mg/kg)	31.4	40.6	42.9	31.2	25
Manganese (mg/kg)	119	161	291	214	25
Minerals affecting availability of copper					*Less than*
Molybdenum (mg/kg)	3.91	3.64	1.97	3.07	1.5
Sulphur (mg/kg)	2246	2862	3111	1677	2000
Iron (mg/kg)	400	221	363	331	500

* Growing and dry stock ** pregnant and lactating stock

Results of the trace element analysis of conserved forages for two of the farms are reported below in Table 2. When looking at the trace elements of interest it can be seen that on the Hereford farm Se levels in forage were similar to those seen in the samples of grazed grass. In Cornwall however, iodine levels reported in conserved forage were around twice the levels seen in the grass samples.

Table 2 Trace element analysis of conserved forage Jan/Feb 2013 (reported on a dry matter basis)

Trace element	Selenium farms		Iodine farms	
	Herefordshire (n=2)	Shropshire	Cornwall (n=3)	Northumberland
	Big bale silage	None fed	Big bale silage	None fed
Selenium (mg/kg)	0.02	*	0.07	*
Iodine (mg/kg)	0.88	*	0.69	*
Cobalt (mg/kg)	0.15	*	0.10	*
Copper (mg/kg)	8.0	*	5.9	*
Zinc (mg/kg)	27.9	*	21.0	*
Manganese (mg/kg)	122	*	83.7	*
Molybdenum (mg/kg)	1.15	*	2.48	*
Sulphur (mg/kg)	1830	*	1486	*
Iron (mg/kg)	397	*	468	*

Individual farm results relating to trace element levels in blood and animal performance are reported below.

Selenium farm - Shropshire

Blood samples

Baseline blood samples of six ewes in September 2012 (Table 3) revealed all to be at or below the recommended level for GSH-Px with mean value of 29.6 U/ml RBCs (range 16.9-50.6). Vitamin B_{12} (cobalt), copper and thyroxine levels were all within the recommended range. Plasma inorganic iodine levels (PII) averaged 5.7 ng/ml (range <5 – 12).

Table 3. Baseline blood samples –September 2012

	Ref range	No.	Mean (s.e.)	Range
GSH-Px (U/ml RBCs)	>50	6	29.6 (6.0)	16.9 – 50.6
Cobalt (Vit B_{12}) (pmol/l)	>188	6	341 (82.0)	217-741
Copper (plasma) (µmol/l)	9-19	6	14.9 (0.66)	13.1-17.0
PII (ng/ml)		6	5.7 (1.28)	<5-12
T4 (nmol/l)	35-75	6	49.7 (6.71)	35.3-80.4

Six ewes from each treatment group were blood sampled 1 February 2013, 121 days after bolusing. All ewes were observed to have GSH-Px levels above the recommended level at this time. Significant differences were seen between treatments with mean levels for the 0.1 Se group significantly lower than the other two groups (Table 4). Figure 1 shows treatment mean and distribution of the values graphically and highlights the range in individual values.

Table 4 GSH-Px levels (U/ml RBCs) – February 2013 – (Day 121 post bolus)

	0.1 Se	0.2 Se	0.3 Se	Significance
No. sampled	6	6	6	
Mean	141.7^{b}	178.7^{a}	183.0^{a}	p =0.04*
St error	11.20	14.12	8.54	
Range	115.1-193.5	122.1-214.2	163.0-222.0	

* Values with the same superscript do not differ significantly.

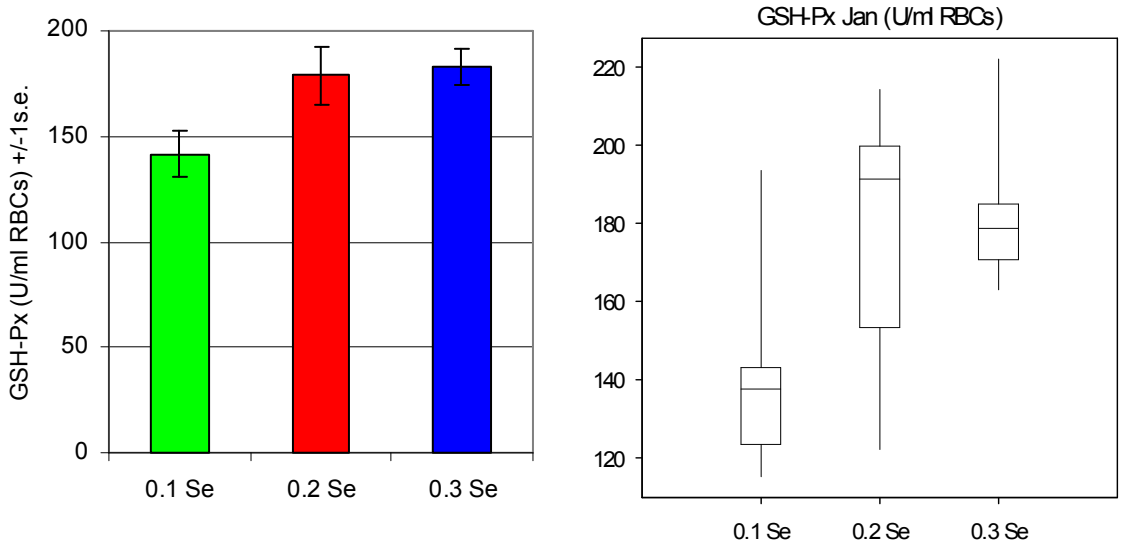

Figure 1. GSH-Px blood samples Jan/Feb 2013. Treatment means and standard errors are shown graphically on left. The box and whisker plot on the right shows the interquartile range and median value within the box with whiskers extending to minimum and maximum values.

Animal performance

Ewe live weight and body condition scores (CS) (Table 5) were unaffected by treatment averaging 65.7 kg and condition score 2.9 at the start of the study. After tupping on grass ewes were moved to stubble turnips from early January where they remained until March. Ewes on all treatments gained some weight and condition during this period averaging 73.6 kg and CS 3.5 in early February. One ewe died between October and February from the 0.2 Se group. Overall pregnancy scanning results averaged 204%; no barren ewes were recorded and although scanning % tended to reduce with increasing Se supplementation significant differences were not observed between treatments.

Table 5. Ewe live weight, body condition scores and pregnancy scanning results

	Overall	0.1 Se	0.2 Se	0.3 Se	s.e.d.	Signif.
Number of ewes	100	34	33	33		
3 October weight (kg)	65.7	64.1	66.1	67.0	1.99	NS
3 October CS	2.9	2.8	3.0	3.1		NS
1 Feb weight (kg)	73.6	73.0	73.6	74.1	2.17	NS
1 Feb CS	3.5	3.4	3.5	3.6		NS
Pregnancy scan (%)	204	212	206	194		NS

Lambing commenced in the third week of March and coincided with prolonged, severe weather conditions. As a result, it was not possible to record information at lambing and the farm dropped out of the study.

In Figure 2 below the trace element profile throughout pregnancy has been plotted for the three treatment groups using the forage results, the Se content of the trial boluses and the declared analysis of any other feeds offered. On this farm Se intakes were below recommended levels before bolusing. All of the treatment boluses boosted Se

supply to recommended levels although this was marginal for the lowest level of supplementation. Se intake whilst ewes were on stubble turnips has been assumed to be the same as on grass but in practice this may not be the case. The daily amount of Se released from the trial boluses was assumed to be constant throughout but in reality is likely to vary, being higher in the first 6-8 weeks and then falling slowly.

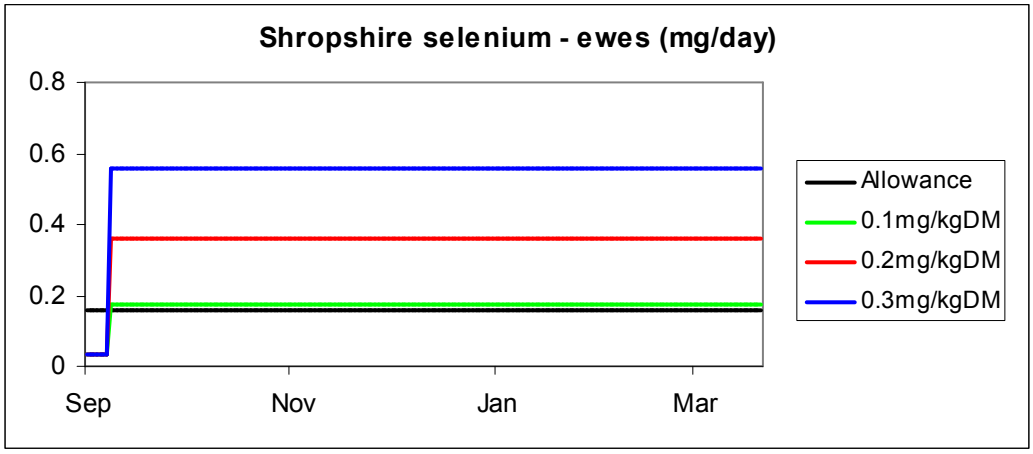

Figure 2. Selenium profile for pregnant ewes (Allowance based on ARC levels)

Selenium farm - Herefordshire

Blood samples

Baseline blood samples of eight ewes in September 2012 revealed around two-thirds to be below the recommended level for GSH-Px with a mean of 49.7 U/ml RBCs (range 21.2-94.8). Vitamin B_{12} (cobalt) and copper levels were above the minimum recommended levels for all animals (Table 6).

Table 6 Baseline blood samples –September 2012

	Ref range	No.	Mean (s.e.)	Range
GSH-Px (U/ml RBCs)	>50	8	49.7 (5.9)	21.2 - 94.8
Cobalt (Vit B_{12}) (pmol/l)	>188	8	500 (80.8)	306 - 694
Copper (plasma) (µmol/l)	9-19	8	14.9 (0.66)	10.6 - 20.9

Six ewes from each treatment group were blood sampled on 22 February 2013, 136 days after bolusing. All ewes were observed to have GSH-Px levels above the recommended level. Although the 0.3 Se group tended to have higher levels than the other two groups (Table 7) significant differences were not observed between treatments (p>0.05). The results are shown graphically in Figure 3.

Table 7 GSH-Px levels (U/ml RBCs) – February 2013 – (Day 136 post bolus)

	0.1 Se	0.2 Se	0.3 Se	Significance
No. sampled	6	6	6	
Mean	163.3	159.8	195.5	*NS*
St error	12.80	14.65	13.15	
Range	110.1-206.7	98.8-183.8	157.0-238.4	

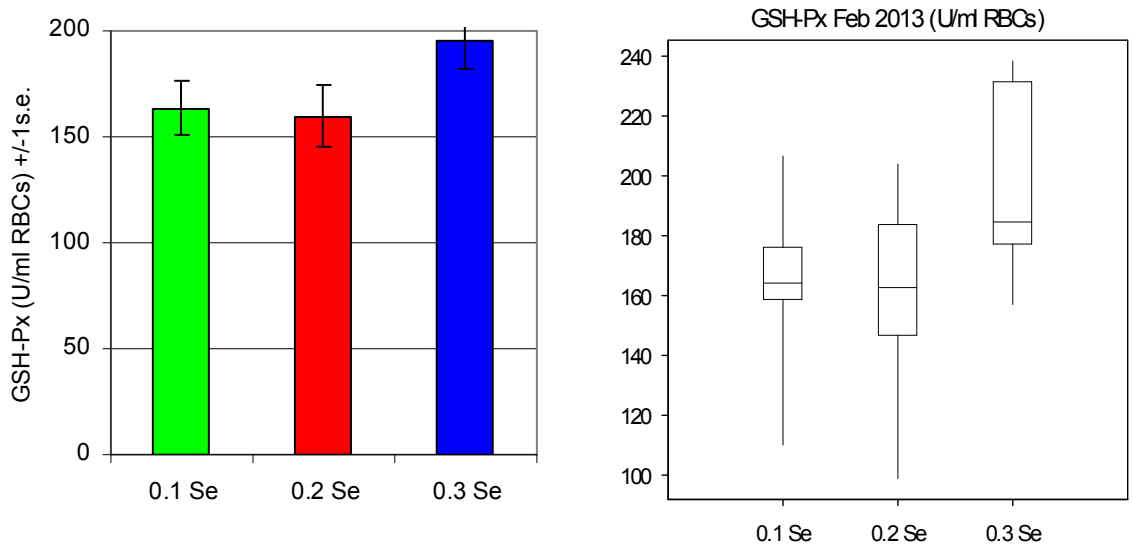

Figure 3. GSH-Px blood samples February 2013. Treatment means and standard errors are shown in graph on left. The box and whisker plot on the right shows the interquartile range and median value with whiskers extending to minimum and maximum values.

Animal performance

Ewe live weight and body condition scores (CS) (Table 8) were unaffected by treatment averaging 68.0 kg and condition score 2.9 at the start of the study. Ewes were grazed on grass throughout pregnancy with supplementary forage offered twice daily from December. Overall, ewes gained a small amount of weight and condition up to February (approx. 6kg and 0.2 CS). Pregnancy scanning results averaged 195%, with three ewes scanned as barren (1 from 0.1 Se and 2 from 0.2 Se groups). As seen on the Shropshire farm although scanning % tended to reduce with increasing Se supplementation significant differences were not observed between treatments. Post-lambing, ewes rearing twins were weighed and condition scored in June/July when their lambs were, on average, 8 weeks old. Significant treatment differences were not observed (p>0.05).

Table 8 Ewe live weight, body condition scores and pregnancy scanning results

	Overall	0.1 Se	0.2 Se	0.3 Se	s.e.d.	Signif.
Number of ewes	150	50	50	50		
Pre-lambing						
9 October weight (kg)	68.0	67.4	68.7	68.0	1.63	NS
9 October CS	2.9	2.9	3.0	2.9		NS
22 Feb weight (kg)	73.8	73.6	74.0	73.6	1.64	NS
22 Feb CS	3.1	3.1	3.1	3.1		NS
Pregnancy scan (%)	195	210	190	184		NS
Post-lambing – restricted to ewes rearing twins						
Number of ewes	98	35	30	33		
Jun/Jul weight (kg)	69.1	70.0	69.2	68.2	1.93	NS
Jun/Jul CS	2.3	2.4	2.4	2.3		NS

Ewes lambed from 5 April with the majority lambing in the first two weeks. Differences in lamb birth weights and lamb vigour between treatments were not evident and losses around birth were reported to be low. For the purposes of assessing subsequent lamb performance a mean birth date of 15 April and birth weight of 4.5 kg for twins and 4.0 kg for triplets (from mature ewes) was assumed. Lambs reared as triplets or by ewes that developed mastitis were excluded from the dataset. Lamb data were analysed by ANOVA with treatment and litter size at birth used as factors. Lamb weights at 8 weeks and at weaning (14 weeks) and the associated DLWGs were unaffected by treatment (p>0.05). Lambs born as twins were however significantly heavier at 8 weeks (25.2 *vs* 22.3 kg, p=0.008) and at weaning (34.6 *vs*. 33.0 kg, p=0.04) than triplet born lambs.

Table 9 Live weight and daily liveweight gains for twin reared lambs

	Overall	0.1 Se	0.2 Se	0.3 Se	s.e.d.	Signif.
Number of lambs assessed	191	68	57	66		
8 week weight (kg)	24.6	24.5	24.5	24.8	1.02	NS
26 Jul weaning weight (kg)	34.3	34.8	33.7	34.3	0.76	NS
DLWG birth to 8wks (g)	324	326	319	326	10.4	NS
DLWG 8wks to weaning (g)	241	251	228	241	17.8	NS
DLWG birth to weaning (g)	291	296	286	290	7.40	NS

In Figure 4 below the trace element profile throughout pregnancy has been plotted for the three treatment groups using the grass and forage results, the Se content of the trial boluses and the declared analysis of any other feeds offered. Se intakes were predicted to be below recommended levels before bolusing but on all treatments the boluses boosted Se supply to at least recommended levels. The daily amount of Se released from the trial boluses was assumed to be constant throughout but in reality is likely to vary, being higher in the first 6-8 weeks and then falling slowly.

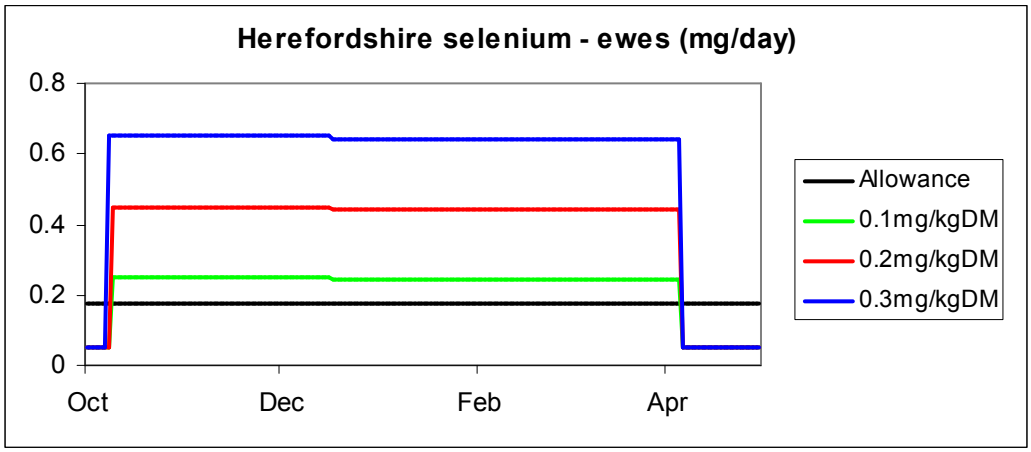

Figure 4. Selenium profile for pregnant ewes (Allowance based on ARC levels)

Iodine farm - Northumberland

Blood samples

Baseline blood samples of ten ewes in July 2012 revealed all to be at or below the recommended level for GSH-Px with mean value of 33.3 U/ml RBCs (range 22.8-52.2) (Table 10). Although mean values for Vitamin B_{12} (cobalt) and copper were in the normal range in both cases three animals were below the minimum recommended levels. Ewes were bolused on 17th October and further blood samples were taken from 15 ewes for PII analysis (five from each treatment group). The majority (13) of samples fell in the range 7-20 ng/ml but two had levels >200 ng/ml.

Table 10 Baseline blood samples – July 2012 (PII Oct 2012)

	Ref range	No.	Mean (s.e.)	Range
GSH-Px (U/ml RBCs)	>50	10	33.3 (2.78)	22.8-52.2
Cobalt (Vit B_{12}) (pmol/l)	>188	10	357 (93.9)	134-1136
Copper (plasma) (µmol/l)	9-19	10	12.8 (2.20)	3.5-21.1
PII (ng/ml)		15	39.9 (18.40)	7->225

Blood samples were taken from five ewes per treatment (plus five non-bolused ewes) 42 days post bolusing and analysed for PII. Supplementing ewes with iodine had significantly raised PII levels above non trial animals (Table 11). Results showed that PII levels increased in line with increasing iodine supplementation, with the 2.0 l group having significantly higher PII levels than the 0.5 l group (p<0.001). PII levels were reported in the range 72 ng/ml to >225 ng/ml across the study ewes.

Table 11 PII levels (ng/ml) – November 2012 – (Day 42 post bolus)

	No bolus*	0.5 l	1.0 l	2.0 l	s.e.d.	Signif.
No. sampled	5	5	5	5		
Mean **	45.2 [c]	107.8 [b]	136.2 [ab]	179.8 [a]	24.72	p<0.001
St error	16.02	15.21	18.46	19.84		
Range	20-108	72-151	78-194	130->225		

* Additional blood samples taken from similar non-study animals in the flock
** Values with the same superscript do not differ significantly.

Ewes were sampled at day 91 to coincide with pregnancy scanning assessments. PII levels had fallen across the treatment groups and the 0.5 and 1.0 l groups were not significantly different to a group of non-bolused ewes (Table 12). Ewes in the 2.0 l group had significantly higher PII levels than all other groups.

Table 12 PII levels (ng/ml) – January 2013 – (Day 91 post bolus)

	No bolus*	0.5 l	1.0 l	2.0 l	s.e.d.	Signif.
No. sampled	8	8	8	8		
Mean **	66.5 [b]	61.4 [b]	78.1 [b]	122.1 [a]	19.11	p=0.014
St error	8.11	6.83	7.60	23.66		
Range	41-110	42-95	39-110	46->225		

* Additional blood samples taken from similar non-study animals in the flock
** Values with the same superscript do not differ significantly.

By day 139 post-bolusing PII levels were similar across the three treatment groups (Table 13). PII levels had altered little for the 2.0l group since the January sampling whilst the 0.5 and 1.0 l groups had increased during this time. Blood samples were also analysed for T4 (thyroxine); results were similar for all treatments (48.6, 48.5 and 56.0 nmol/l for 0.5, 1.0 and 2.0 l respectively) with all animals within the reference range (35-75 nmol/l). PII results are shown graphically below in figures 5 and 6.

Table 13 PII levels (ng/ml) – March 2013 – (Day 139 post bolus)

	0.5 l	1.0 l	2.0 l	s.e.d.	Signif.
No. sampled	5	5	5		
Mean	96.6	108.0	117.8	22.72	NS
St error	15.97	10.51	22.25		
Range	52-146	83-136	79-200		

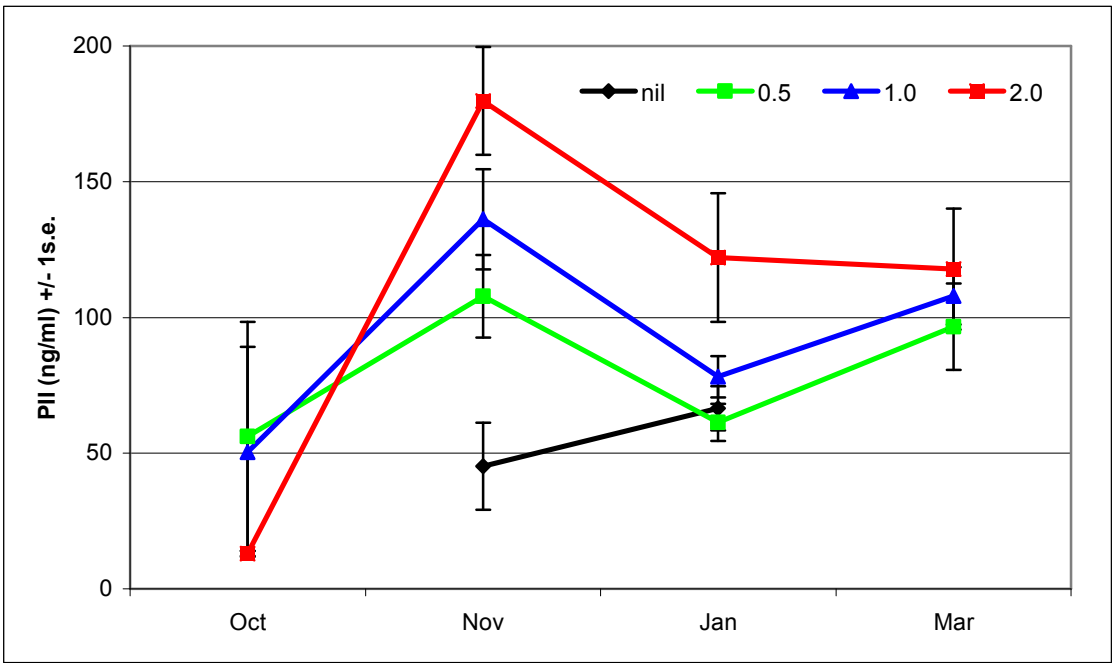

Figure 5. Plasma inorganic iodine levels (PII) by treatment

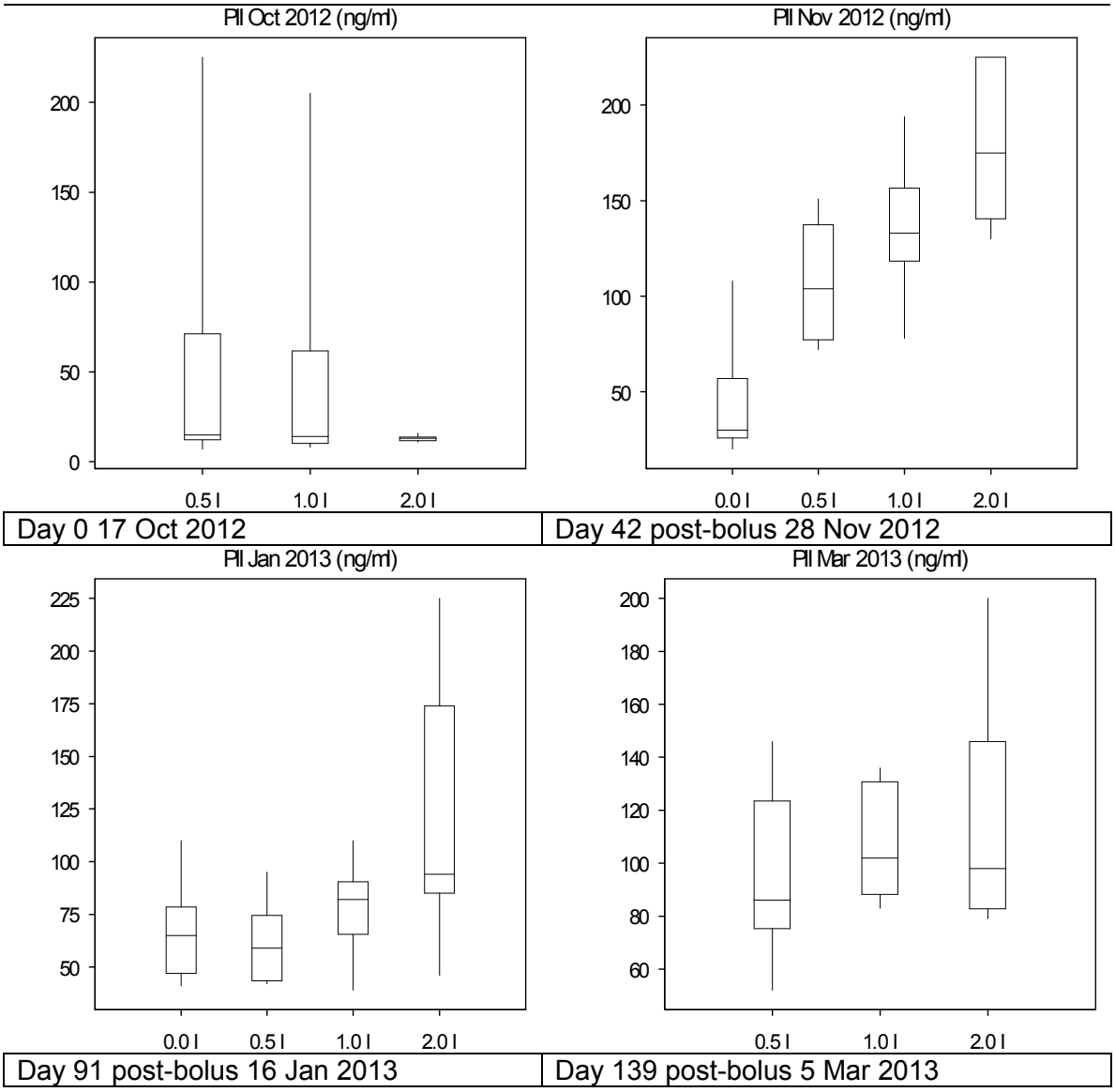

Figure 6. Series of box and whisker plots displaying the distribution of PII data on each of the sampling days, interquartile range and median value in the boxes with whiskers extending to minimum and maximum values.

Animal performance

Ewe live weight and body condition scores (CS) (Table 14) were unaffected by treatment at any point during pregnancy averaging 70.9 kg and condition score 3.1 at the start. Ewes remained on grazing throughout pregnancy with triplet and some twin-bearing ewes being supplemented with oats in late pregnancy. Ewes on all treatments lost weight and condition during pregnancy averaging 62.3 kg and CS 2.4 at four weeks pre-lambing. Pregnancy scanning results averaged 164% and did not differ significantly between treatments. In total eight ewes scanned as barren (2 from 0.5 l, 4 from 1.0 l and 2 from 2.0 l groups). Post-lambing, ewes were weighed and condition scored in May when their lambs were approximately 5 weeks old. Significant treatment differences were not observed ($p>0.05$).

Table 14 Ewe live weight, body condition scores and pregnancy scanning results

	Overall	0.5 l	1.0 l	2.0 l	s.e.d.	Signif.
Number of ewes	102	34	34	34		
Pre-lambing						
October weight (kg)	70.9	69.7	72.1	70.9	1.75	NS
October CS	3.1	3.1	3.1	3.1		NS
January weight (kg)	64.8	63.3	65.8	65.1	1.70	NS
January CS	2.8	2.8	2.9	2.8		NS
March weight (kg)	62.3	61.4	64.0	61.4	1.77	NS
March CS	2.4	2.4	2.4	2.4		NS
Pregnancy scan (%)	164	171	171	150		NS
Post-lambing						
Number of ewes	90	32	28	30		
May weight (kg)	54.4	53.5	55.8	54.0	1.55	NS
May CS	2.1	2.1	2.1	2.0		NS

Ewes lambed outdoors from late March. Differences in lamb birth weights and lamb vigour between treatments were not observed and although no lambs were submitted for examination of the thyroid there were no signs of iodine deficiency. For the purposes of assessing subsequent lamb performance a mean birth date of 11 April and birth weight of 3.8 kg for singles and 3.2 kg for twins was assumed. Lamb data were analysed by ANOVA with treatment and litter size at birth used as factors. Lamb weights at 5 weeks and at weaning (approximately 16 weeks) and the associated daily gains were unaffected by treatment (p>0.05) (Table 15). Single lambs grew significantly faster than twins between May and July (315g *vs* 296g, p=0.008) and were heavier at weaning (36.9 *vs* 35.1 kg, p=0.038). In the 2013 season no symptoms of iodine deficiency were seen in any of the groups and the decision was taken to leave the flock unsupplemented for the 2014 lambing crop.

Table 15 Lamb performance data by treatment

	Overall	0.5 l	1.0 l	2.0 l	s.e.d.	Signif.
Number of lambs monitored	143	46	54	43		
16 May 5 wk weight (kg)	12.5	12.5	12.7	12.2	0.56	NS
31 Jul weaning weight (kg)	35.5	35.7	36.0	34.7	0.88	NS
DLWG birth to 5 wks (g)	295	294	304	285	18.0	NS
DLWG 5wks to weaning (g)	301	304	303	295	7.1	NS
Overall DLWG to weaning (g)	301	302	306	293	8.3	NS

In Figure 7 below the trace element profile throughout pregnancy has been plotted for the three groups using the forage results and I content of the trial boluses. Iodine intakes from forage were predicted to be slightly above requirements in this flock and the boluses boosted predicted intakes well above recommended levels. The daily amount of I released from the trial boluses was assumed to be constant throughout but in reality is likely to vary, being higher in the first 6-8 weeks and then falling slowly.

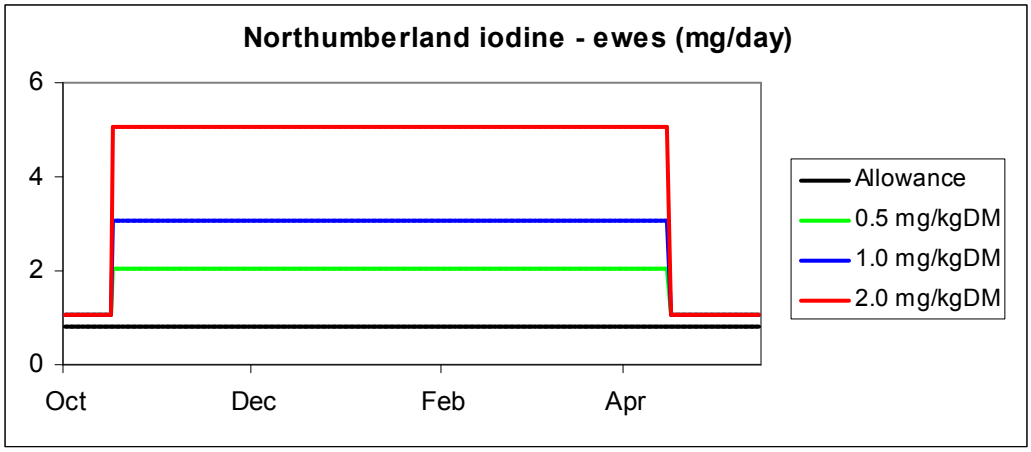

Figure 7 Iodine profile for pregnant ewes (Allowance based on ARC levels)

Iodine farm - Cornwall

Blood samples

Baseline blood samples of six ewes in September 2012 (Table 16) revealed all ewes to be within the recommended range for GSH-Px, Vitamin B12 (cobalt) and copper. Plasma inorganic iodine levels (PII) were assessed on a pooled sample averaging 14.0 ng/ml.

Table 16 Baseline blood samples – September 2012

	Ref range	No.	Mean (s.e.)	Range
GSH-Px (U/ml RBCs)	>50	5	233 (21.8)	186 - 294
Cobalt (Vit B12) (pmol/l)	>188	6	619 (131.5)	422 -1273
Copper (plasma) (µmol/l)	9-19	6	11.3 (0.6)	9.9 -13.2
PII (ng/ml)		6	14.0	Pooled sample

By day 59 post-bolusing PII levels had risen for all treatments (Table 17). Overall, results just failed to meet statistical significance at the 5% level (p=0.058) but PII for 0.5 l ewes was lower than the 2.0 l group.

Table 17 PII levels (ng/ml) – 30 November 2012 – (Day 59 post bolus)

	0.5 l	1.0 l	2.0 l	s.e.d.	Signif.
No. sampled	6	6	6		
Mean *	25.8 [b]	61.8 [ab]	66.7 [a]	16.98	*p*=0.058
St error	2.85	10.36	17.81		
Range	15-32	27-97	<5-119		

* Values with the same superscript do not differ significantly.

At day 119 post bolusing PII levels had fallen across all treatments with several results (7 out of 18) below the minimum reporting level. Significant differences were not observed between treatments. In addition to PII blood samples were analysed for GSH-Px in November and January to ensure Se levels were adequate. On both occasions GSH-Px levels were well above the minimum recommended (overall average 253.0 and 300.7 U/ml RBCs in Nov and Jan respectively) and were similar for all treatments. Plasma inorganic iodine results are shown graphically in Figures 8 and 9.

Table 18 PII levels (ng/ml) – 29 January 2013 – (Day 119 post bolus)

	0.5 I	1.0 I	2.0 I	s.e.d.	Signif.
No. sampled	6	6	6		
Mean	12.3	19.7	17.2	14.77	NS
St error	5.06	12.18	12.38		
Range	<5-36	<5-80	<5-79		

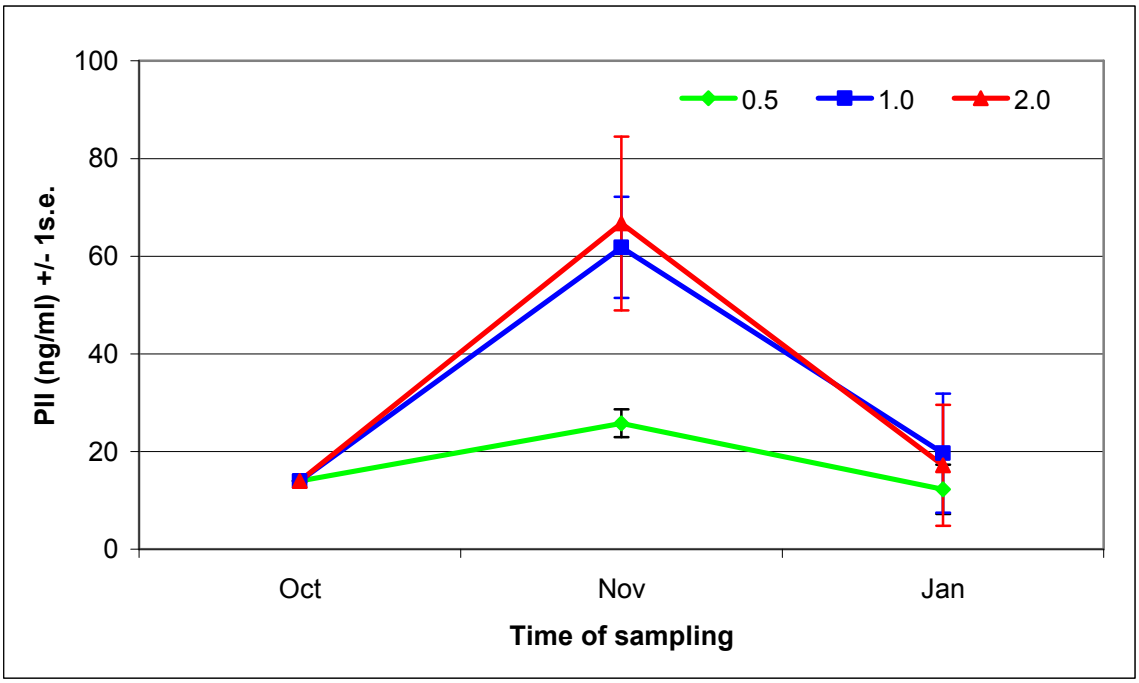

Figure 8. Plasma inorganic iodine levels – Cornwall

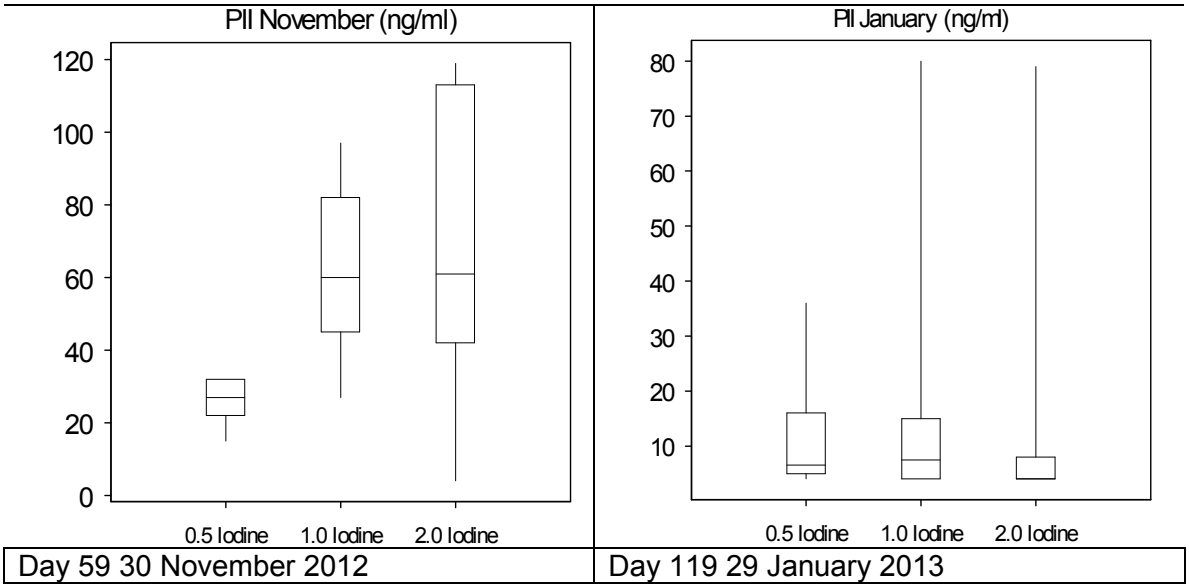

Figure 9. Box and whisker plots displaying the distribution of the data in November and January, interquartile range and median value displayed in the box with whiskers extending to minimum and maximum values.

Animal performance

Ewe live weight and body condition scores (CS) (Table 19) were unaffected by treatment averaging 74.5 kg and condition score 3.0 at the start of the study. Ewes were tupped at grass and supplemented with SBP and high energy feed blocks. After tupping ewes received supplementary forage (hay) at grass before housing on big bale silage/haylage. Housed ewes were winter shorn in January. In late pregnancy ewes were supplemented with a home-mixed ration based on barley, oats, SBP, rapeseed meal and soya with added minerals building up to around 0.8 kg at lambing for twin bearing ewes. Ewes on all treatments gained weight to February and maintained condition score averaging 87.3 kg and CS 2.9. One ewe died from pasteurella in December from 0.5 l group. Overall pregnancy scanning results averaged 223% and were similar for all groups with only one barren ewe recorded in the 0.5 l group.

Table 19 Ewe live weight, body condition scores and pregnancy scanning results

	Overall	0.5 l	1.0 l	2.0 l	s.e.d.	Signif.
Number of ewes	90	30	30	30		
Pre-lambing						
2 October weight (kg)	74.5	73.9	74.2	75.5	1.36	NS
2 October CS	3.0	2.9	3.0	3.1		NS
18 January weight (kg)	78.5	78.1	78.0	79.3	1.43	NS
18 January CS	2.8	2.8	2.8	2.9		NS
21 February weight (kg)	87.3	87.9	86.3	87.7	1.91	NS
21 February CS	2.9	2.8	2.9	2.8		NS
Scanning % *	223	228	220	220		NS
Post-lambing						
Number of ewes	77	24	25	28		
7 August weight (kg)	78.6	79.2	77.5	79.2	2.16	NS
7 August CS	2.8	2.8	2.8	2.7		NS

* excluding one ewe died before scanning in 0.5 l group.

Ewes lambed indoors from mid mid-March and were turned out to grass within 24-48 hrs. Lambs were tagged and birth date recorded before turn out. Differences in lamb vigour, birth weight or lamb losses been treatments were not observed and for the purposes of assessing subsequent lamb performance a standard birth weight of 5.0 kg was assumed. Lambs that were reared artificially were excluded from the analysis. Lamb live weights in May and June and associated growth rates were similar for all treatments ($p>0.05$). At weaning (approximately 18 weeks) lambs from the 0.5 l group tended to be lighter than those from the other two groups although this just failed to meet statistical significance at the 5% level ($p=0.056$). Overall gains to weaning were similar for all treatments.

Table 20. Lamb performance data by treatment

	Overall	0.5 I	1.0 I	2.0 I	s.e.d.	Signif.
Number of lambs	149	48	53	48		
May weight 5 wks (kg)	18.4	18.1	18.4	18.7	0.50	NS
Jun weight 13 wks (kg)	33.6	32.8	33.9	34.2	0.84	NS
Weaning weight (kg)	38.5	37.2	39.1	39.0	0.87	P=0.056
DLWG to May (g)	362	355	365	366	14.0	NS
DLWG May – Jun (g)	267	263	270	269	9.0	NS
DLWG birth to Jun (g)	305	301	308	307	8.7	NS
DLWG Jun-weaning	135	127	145	131	10.4	NS
DLWG to weaning (g)	260	253	265	260	7.0	NS

In Figure 10 the trace element profile for iodine throughout pregnancy has been plotted for the three groups using the grass and forage results, I content of the trial boluses and the declared analysis of concentrate feeds and other supplements. The daily amount of I released from the trial boluses was assumed to be constant throughout but in reality is likely to vary, being higher in the first 6-8 weeks and then falling slowly. Iodine intakes from forage were predicted to be below the recommended levels for pregnant and lactating ewes prior to bolusing. Following bolusing all treatments met or exceeded recommended levels. Iodine intake was increased further during tupping through supplementation by SBP and access to high energy feed blocks. In late pregnancy ewes on all treatments received additional iodine in the home mixed ration and from a single oral dose of potassium iodide in February (4 weeks pre-lambing). The oral dose contained very high levels of iodine (285 mg) and has therefore been allocated over a ten day period in the figure below.

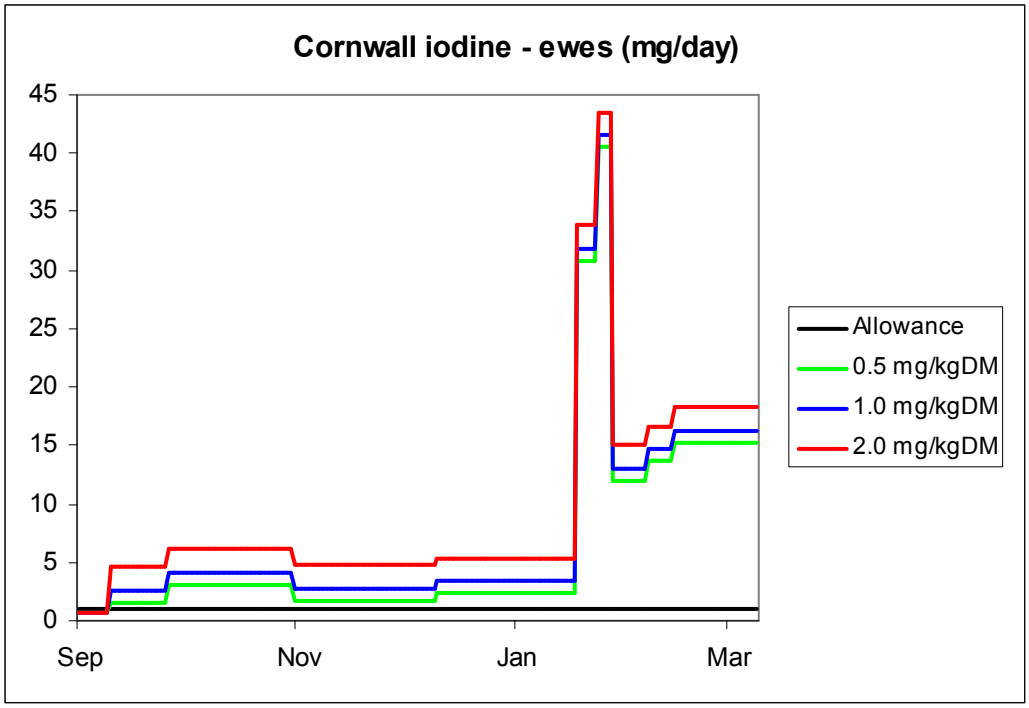

Figure 10 Iodine profile for pregnant ewes (Allowance based on ARC levels)

Summary of results

For this study four farms were selected on the basis of historic trace element problems. Two had a history of selenium deficiency (Hereford and Shropshire) and the farms in Cornwall and Northumberland were selected by the farm vets as having clinical signs of iodine deficiency. On the Northumberland farm, goitre and thyroid hyperplasia had been seen in stillborn lambs, whilst on the Cornish farm, neonatal goitre had been observed in stillborn calves.

Samples of grass and conserved forage were collected at the start of the study from all four farms. For the farms with a history of low selenium, the sampling confirmed that selenium concentrations in the forage were below the recommended levels for ruminants. However, the iodine concentrations in the forage from the other farms were within the normal range. Soil analyses were not undertaken.

Results from the selenium study

On the selenium farms baseline blood sampling of ewes confirmed low or marginal GSH-Px concentrations at the start of the study. GSH-Px concentrations in the Shropshire flock averaged 30 U/ml RBC with only one animal reaching the reference level of >50 U/ml RBC. The mean value in the Herefordshire flock was just adequate at 50 U/ml RBC but in the range 21 – 95 U/ml RBC. Selenium supplementation at 0.1, 0.2 and 0.3 mg per kg DM all significantly increased the GSH-Px levels on both farms with the treatment means across the two flocks all above 140 U/ml RBC at 120-136 days post bolus and the lowest value was 99 U/ml RBC. While the 0.2 mg and 0.3 mg Se treatments resulted in significantly higher GSH-Px concentrations than the 0.1 mg treatment on the Shropshire farm, on the Herefordshire farm there was not a significant difference between the 3 different selenium treatments.

Ewe weights and body condition during pregnancy were unaffected by the level of selenium supplementation pre-tupping. On the Herefordshire farm, no difference in ewe productivity was observed between the three treatment groups. Data on the productivity of ewes on the Shropshire farm is not available as the farm withdrew from the project.

Differences in lamb vigour and lamb losses between treatments were not observed on the Herefordshire farm and clinical signs suggestive of Se deficiency were not seen.

Results from the iodine study

On the two iodine farms, PII concentrations were considered to be low (AHVLA, 2013) at the start of the study, and increased after bolus administration in both cases. In Northumberland the mean PII concentration at the start was 40 ng/ml and bolus supplementation increased the PII concentration in proportion to increasing levels of iodine in the bolus. At day 42 after bolus administration, the mean PII concentrations of the ewes that received a bolus (0.5, 1.0 or 2.0mg/kg DM) were all above 105 ng/ml, the level considered normal by the AHVLA for iodine supplemented animals (AHVLA 2013). By day 91 only the ewes in the 2.0mg group had a mean PII level of >105ng/ml. At day 139, the mean PII levels for the 0.5 and 1.0 mg groups were higher than on day 91. The increase in PII values between day 91 and day 139 may be due to additional iodine in supplementary feed given pre-lambing, or may simply reflect variation between ewes, as the same ewes were not sampled on each occasion. At day 139, thyroxine levels were also measured. For all the treated ewes, the thyroxine level was within the reference

range with the mean values for the three groups (0.5, 1.0 and 2.0mg) being very similar (48.5, 48.6 and 56.0 nmol/l respectively).

On the Cornish farm, the PII concentration of a pooled sample at the start of the study was low at 14 ng/ml. Following bolus administration, the mean PII concentration in supplemented ewes did not reach the 105ng/ml threshold at either sampling point (days 59 and 119 post bolus). At day 59 post bolus, mean PII levels for the three treatment groups (0.5, 1.0 and 2.0 mg I) were 25.8, 61.8 and 66.7 ng/ml respectively. At day 119 post bolus, the mean PII levels for the three groups were all low (less than 20 ng/ml in all treatment groups).
As in the selenium study, ewe weights and body condition during pregnancy were unaffected by the level of iodine supplementation pre-tupping; differences in lamb vigour and lamb losses between treatments were not observed in stock on either of the trial farms and no clinical signs suggestive of iodine deficiency were seen.
Lamb performance was unaffected by the treatment regime in the ewes. There was a tendency for lambs on the low iodine (0.5 mg I) treatment to be lighter at weaning on the Cornish farm, but the difference was not statistically significant**.**

Discussion and conclusions

Selenium

The GSH-Px results indicate that, on these two farms under the conditions of this study, supplementation with a bolus providing 0.1 mg Se/kgDM was sufficient to maintain GSH-Px levels well above the reference range for more than 120 days. This is also supported by the lack of any difference in the productivity of the ewes on the three selenium treatments on the Herefordshire farm. Therefore, the conclusion is that there appeared to be no advantage in supplementing with more than 0.1 mg Se/kgDM under the current nutrition regime on these farms.

However, selenium requirements vary with diet (for example, with varying vitamin E levels) and with the level of production, so there may be circumstances where a higher GSH-Px level, and consequently a higher level of selenium supplementation, may be beneficial.

Iodine

The results from the iodine study are more difficult to interpret. The PII concentrations on both farms were elevated following administration of the boluses, but the duration of the elevation of PII concentrations was less than expected. Also, the mean PII concentrations recorded on the Cornish farm were not as high as expected

Iodine sufficiency or deficiency in grazing livestock is much more difficult to assess than selenium. The main reason is that the diagnostic tests are poorer and more difficult to interpret. Also, whereas there tends to be a fairly consistent relationship between selenium levels in soil and selenium status in grazing animals, with iodine the relationships are more complex. Selenium is readily taken up by plants (some plants accumulate selenium), whilst soil to plant transfer rates of iodine are low. Iodine levels in herbage depend on the iodine absorbed by the roots from the soil and on iodine absorbed by the leaves from the atmosphere and rainfall. Topsoil generally contains more iodine than herbage, so where grazing animals consume significant amounts of soil, this may have a significant effect on the animals' iodine status.

A further problem with investigating iodine sufficiency is in measuring iodine status in the animal. Approximately 80% of iodine is in the thyroid gland, and it is not practical to assess thyroid iodine content in the live animal. Thyroid size is used as an indicator of neonatal iodine deficiency, but an obviously enlarged thyroid or goitre only occurs when the deficiency is severe and of several months duration. Histopathological examination of neonatal thyroids, and measuring thyroid iodine content is a more sensitive way of detecting iodine deficiency, but is expensive.

Iodine in the blood is mostly in the form of the thyroid hormone, thyroxine or T4. Unbound iodine in the blood can be measured by PII assay. PII is accepted as a sensitive indicator of iodine intake during the previous 2 to 3 days. It is considered to be a measure of nutritional iodine status and not the animal's iodine status.

The results from the iodine study show that PII increased following bolus administration on both farms, but that PII concentrations were not maintained for the expected duration of the boluses. The commercially available boluses claim to release selenium, cobalt and iodine for 180 days, but PII results on these farms suggest that by 3 months post administration boluses with three different iodine levels had a limited effect on the

Cornish farm and only the 2mg bolus (the commercial variant) was still effective on the Northumberland farm. On the Cornish farm at day 119 post bolus the mean PII concentrations for the three treatment groups were all low (AHVLA, 2013) at less than 20 ng/ml. Whilst on the Northumberland farm, only the 2 mg bolus group had a mean PII concentration of greater than 105 ng/ml. Possible reasons for the apparent short duration of efficacy of the boluses include:

- bolus expulsion
- the bolus running out of iodine
- formation of an insoluble coating making the iodide inaccessible (but this is unlikely with an eroding style bolus)
- reaction with other elements causing the iodine to be in a form that is still available to the animal, but not detected by the PII assay
- A block on the absorption of the iodine from the gastro-intestinal tract.

More work is needed to determine whether the boluses had eroded away, run out of iodine, or deposits on the bolus were making the iodine unmeasurable by PII. The use of labeled iodine would help here.

On the Cornish farm, the mean PII concentration in iodine supplemented ewes did not reach the 105 ng/ml threshold at either sampling point (days 59 and 119 post bolus). The highest mean PII values were recorded on day 59 post bolusing, when concentrations for the three treatment groups (0.5, 1.0 and 2.0 mg I) were 25.8, 61.8 and 66.7 ng/ml respectively. However, as PII is considered to be an indicator of iodine intake over the previous 2 to 3 days only it is not possible to extrapolate PII levels between sampling points. The divergence of the results in Cornwall compared to Northumberland was strange. The same batches were used at both sites and while it is possible that a rapid early release occurred between day 0 and day 59 in Cornwall there is no evidence that this occurred in Northumberland.
The failure of PII concentrations to rise as high as expected on the Cornish farm raises the question as to whether the current interpretation of PII concentrations proposed by AHVLA is suitable, or whether iodine status is adequate at lower PII concentrations than suggested. It was planned to measure thyroxine levels on both farms 3 months after administration of the boluses, and it is unfortunate that this was not done on the Cornish farm. Measuring thyroxine may have helped to show whether or not the apparently low PIIs were affecting thyroid iodine levels and the formation of thyroxine.
Also, no lamb thyroid glands were examined. A study in cattle where heifers were fed an iodine deficient diet for the last few months of pregnancy found no differences in survival between calves born to heifers fed an iodine deficient diet and heifers fed an iodine sufficient diet. However, the thyroids of both the heifers fed the deficient diet, and their newborn calves, showed clinicopathological and pathological changes consistent with iodine deficiency (McCoy et al, 1997).
In the study on pregnant heifers, the mean PII concentrations of the heifers fed an iodine sufficient diet plateaued at 55 to 60 ng/ml. This is similar to the mean PII concentrations recorded on the Cornish farm, and these findings suggest that PII concentrations of this magnitude in pregnant sheep or cattle may indicate adequate iodine intake.
This study has shown wide variation in PII values between individual ewes, with values varying between 7 and >225 (the upper limit of the assay) between 5 ewes. PII is an expensive assay to run, so it is common practice to pool samples for testing. The wide variation between individual values suggests that pooling may not give an accurate reflection of PII status when only a small number of individual samples contribute to the pool. Once again, further study may be needed to help to define a protocol for pooling plasma.

A further reason for measuring thyroxine as well as PII that has not been explored in this study, is the presence of goitrogens. These are substances that interfere with the absorption of iodine, either from the gastro-intestinal tract, or more commonly, with the uptake of iodine by the thyroid gland. The presence of goitrogens influences the requirements for iodine, with the effects of most goitrogens being overcome by giving additional iodine. When goitrogens that affect utilization of iodine in the thyroid gland are present, PII concentrations may appear adequate, but thyroxine levels would fall. This reinforces that measuring PII and thyroxine together provides a more accurate indication of an animal's iodine status than using PII or thyroxine alone.

Conclusions

The primary objective of this study was to refine and confirm the level of selenium and iodine supplementation for breeding ewes by evaluating the effect of differing levels of selenium and iodine supplementation on performance. As stated above, the results need to be treated with caution because the data is limited to only 1 farm for selenium and 2 for iodine.

However, in this study pre-tupping administration of a sustained release intra-ruminal bolus providing 0.1 mg selenium per kg DM was found to provide adequate levels of selenium on a known selenium deficient farm, with no observed benefits in supplementing with 0.2 or 0.3 mg selenium per kg DM.

For iodine, the results have raised more questions than answers. The duration of effect of the boluses, as measured by PII concentrations was less than expected on both farms. Also, PII concentrations did not rise as high as expected on one farm. Questions are raised as to whether the current guidelines for the interpretation of PII are suitable for assessing the adequacy of iodine intake in pregnant ewes.

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