European Innovation Partnership (EIP) Wales

Implementing advanced nutritional management in the Welsh sheep industry

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Introduction

Optimal nutrition underpins effective and efficient livestock production and for Welsh sheep farmers production relies heavily on the utilisation of grazed and conserved grass. Indeed, recent industry advice has focussed on improving efficiency by reducing added feed costs and focusing on utilising grass and conserved forage availability as much as possible (1). However, in order for the sheep to perform efficiently, grass or conserved forage may not provide all the nutritional elements required and it is widely acknowledged that in many areas and on many farms the grass may over or undersupply various nutrients to the sheep (2-4). In addition, different breeds of sheep assimilate, store and use individual nutritional elements differently and the nutritional composition of the grass will alter during the year and between years. As such nutritional supplementation is widely practiced in order to try and improve productivity and address these perceived imbalances.

For instance, many farms will try and optimise the timing of lambing to coincide with an increase in grass growth in spring. This can be challenging and is obviously weather dependent, and there is a temptation to lamb earlier if possible, to try and take advantage of the expected elevation in lamb prices earlier in the summer. Consequently, many farms may require supplementation of their pregnant ewes with a high energy and/or protein source close to lambing if the available forage cannot meet the needs of the sheep, although sometimes this additional feeding may be seen more as insurance than required.

Nutritional planning may be reactive to a clinical or production problem e.g. energy supplementation carried out when pregnancy toxaemia is diagnosed or now more typically as a planned management strategy. For some nutritional components a clinical disease or syndrome may not be observed overtly, however farmers are generally moving to optimise production where possible and this includes preventive strategies to optimise nutrition.

In the UK as a whole, the decision to supplement may be made arbitrarily by the farmer based on a perceived nutritional deficiency, historic practice, or based on observations and tests of the grass, the soil or the sheep. However, the only way to assess whether the sheep are supported nutritionally at any one point is to investigate markers within the sheep and compare them to established norms. Traditionally in the UK this has been done through a combination of body condition scoring (BCS) together with the farmer's vet taking blood samples from a sample of sheep, and then assessing the concentration of various nutritional elements or markers within them. For many situations this is likely to provide useful information, for example assessing the energy status of pregnant ewes, however, if the goal is for optimal production and preventive nutrition, then for some trace element markers this is probably inadequate in that blood concentrations can respond to diet changes within days and may also

be influenced by other disease processes for example parasitic gastroenteritis, fascioliasis or some other inflammatory process(5).

In New Zealand, the trace element status of some sheep flocks is now monitored routinely using liver tissue biopsies sometimes with additional blood samples (6, 7). The liver tissue sample provides different information to blood in that it provides a much longer-term historical estimation of the status of some trace elements, particularly copper. For example, some elements, including copper, are excreted through the liver and the concentration adjusts much more slowly over several months compared to within blood, and therefore liver copper concentrations allow a better understanding of historic supply. This information coupled with an understanding of the expected nutritional demands of the sheep, together with an understanding of the potential available supply can enable more proactive planning for nutritional adjustment.

Together with this, blood analysis is still useful, in that it can provide short term information indicative of current supply and response as well as information regarding element competition, and indeed the two samples taken in parallel provide the most comprehensive indication of historic and current trace element status and provide the best information to formulate management advice for future dietary adjustments (5-7). Moreover, in some progressive areas of the UK this process is now being used successfully in dairy cattle, which may be at greater risk of *over* supplementation (8).

Aim of the project

In this project we aimed to utilise a comprehensive approach to nutritional planning in the Welsh sheep context, with a focus on managing trace elements proactively in breeding ewes. We aimed to use blood and liver tissue samples taken in tandem, together with an analysis of the available forage in order to explore the benefits to sheep farmers in developing targeted feeding and supplementation plans. We chose to focus on ewes exploring their nutritional needs from before pregnancy through to lambing.

Methodologies employed

Throughout this project body condition scoring was utilised based on the method described by Russel (9). All blood samples were obtained via jugular venepuncture into plain, heparinised and EDTA tubes prior to analysis. Liver biopsies were carried out according to the method described by Sargison (6). Energy, protein and trace element analyses were carried out at NUVetNA laboratories, the University of Nottingham, and haematology and parasite analyses carried out at Wern Veterinary Surgeons. We would have liked to investigate the element iodine in this project as it has important effects on fertility and lamb survival, however due to technical issues it was unfortunately impractical to conduct these analyses in addition to those detailed in this report.

Some of the blood analyses used are not routinely carried out in sheep practice, e.g. the measurement of superoxide dismutase, however they were utilised here as it was considered that the depth of information provided would enable a more detailed and nuanced understanding of the status of the ewes and therefore a more balanced approach to diagnosis, management and supplementation.

The liver biopsy technique is also unusual in the Welsh sheep context and so a brief description is provided here for illustrative purposes. This technique has been shown to be safe and may be carried out by a veterinary surgeon when clinically justified. The technique is challenging and does not always yield a sample in every case. In this project a suitable sample was obtained in 200/233 attempts (85.8%) with success frequency and tissue yields improving over time. In this project 4 sheep died shortly after biopsy (1.7%). Three were available for immediate postmortem examination and all three had previously undetected comorbidities. One sheep had a severe pneumonia (various others in the group were then noted to be coughing some days later and managed accordingly) and two were noted to have liver fluke infection. These comorbidities were very likely to have contributed to the death of the sheep when subsequently biopsied. As a result, farmers are now advised to either test or treat the sample ewes for fascioliasis well in advance of undertaking liver biopsies.

Objectives

The project was structured to address several specific objectives in order to address the aim:

Objective 1 – diagnosis of deficiencies/areas to optimise and formulation of nutritional advice

a) *Determine the energy and trace element status of a sample of breeding ewes at the start of the breeding season for each participant farm.*
b) *Determine the nutritional value of the available forage.*
c) *Formulate nutritional planning advice based on these data and knowledge of the farm.*

To achieve this objective, background data collection and sampling of the ewes and forage was carried out on the 12 farms between 07/09/2018 and 08/10/2018 by JA. Reports were then generated for all the farmers and advice given (by JA) on the basis of the available data.

The farms in the OG were all commercial sheep farms including a range of breeds and operating in a variety of contexts (Table 1).

Farm ID	Land type	Total number hectares	Approximate number ewes	Breeds
1	Improved upland	110	500	Llyn x Texel; Aberfield
2	Hill and lowland	303.5	350	Exlana
3	Lowland/improved upland	64.7	200	Texel x; Beulah; Mules
4	Lowland/improved upland	101.2	450	Welsh mules
5	Lowland/improved upland	65	200	Mule
6	Improved upland	202.3	1000	Crossbreds
7	Improved upland/Hill	202.3	900	Welsh; BFL x Texel
8	Hill/Improved upland	81	360	Welsh
9	Hill/improved upland	283.3	700	Welsh
10	Hill and improved upland	353	900	Welsh; Cheviot x Welsh
11	Hill/improved upland	320	1060	Welsh; Romney x Welsh
12	Improved upland	81	220	Texel cross

Table 1: Details of the sheep farms included in this project.

General health (2018)

Each farmer presented a random sample of 20 ewes from the flock for inspection. These were body condition scored and a general assessment of their health was made. In 2018, the summer period had been unusually dry and there was a generalised and severe lack of forage on many of the farms. The body condition scores (BCS) for the ewes was generally below a pre-tupping target for all the farms

except farm 10 (Figure 2). For hill flocks, a pre-tupping target of 2.5 was desired and for lowland flocks 3.5 (10). Farm 10 was a hill flock and whilst BCSs were around 2.5 or higher some sheep were still below this value. The BCSs for some of the farms was considered critical e.g. farms 2, 3, 4 and 9 with particular concern for farms 2 and 4.

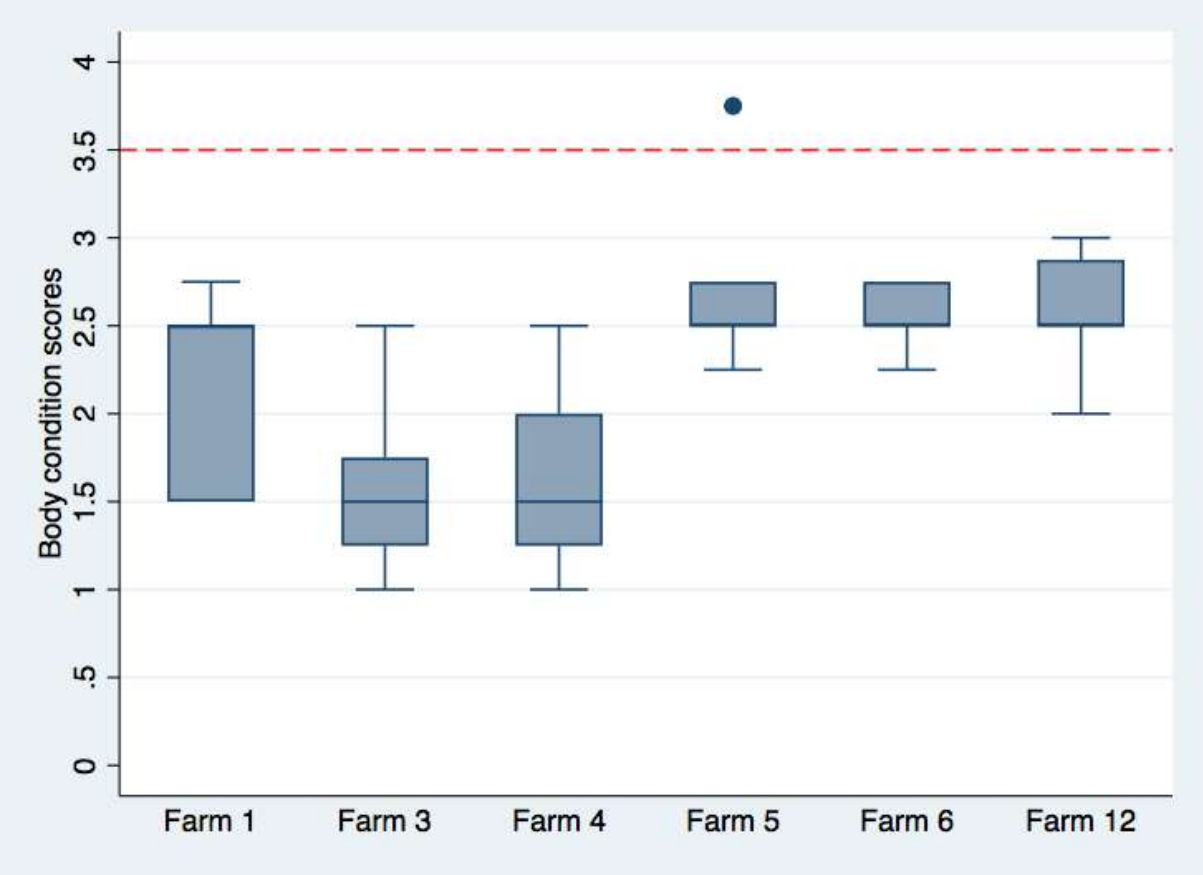


Figure 1: Box and whisker plots of body condition scores for ewes from lowland/improved upland farms presented pre-tupping in Autumn 2018. The red dashed line reflects the target BCS for ewes for these farms.

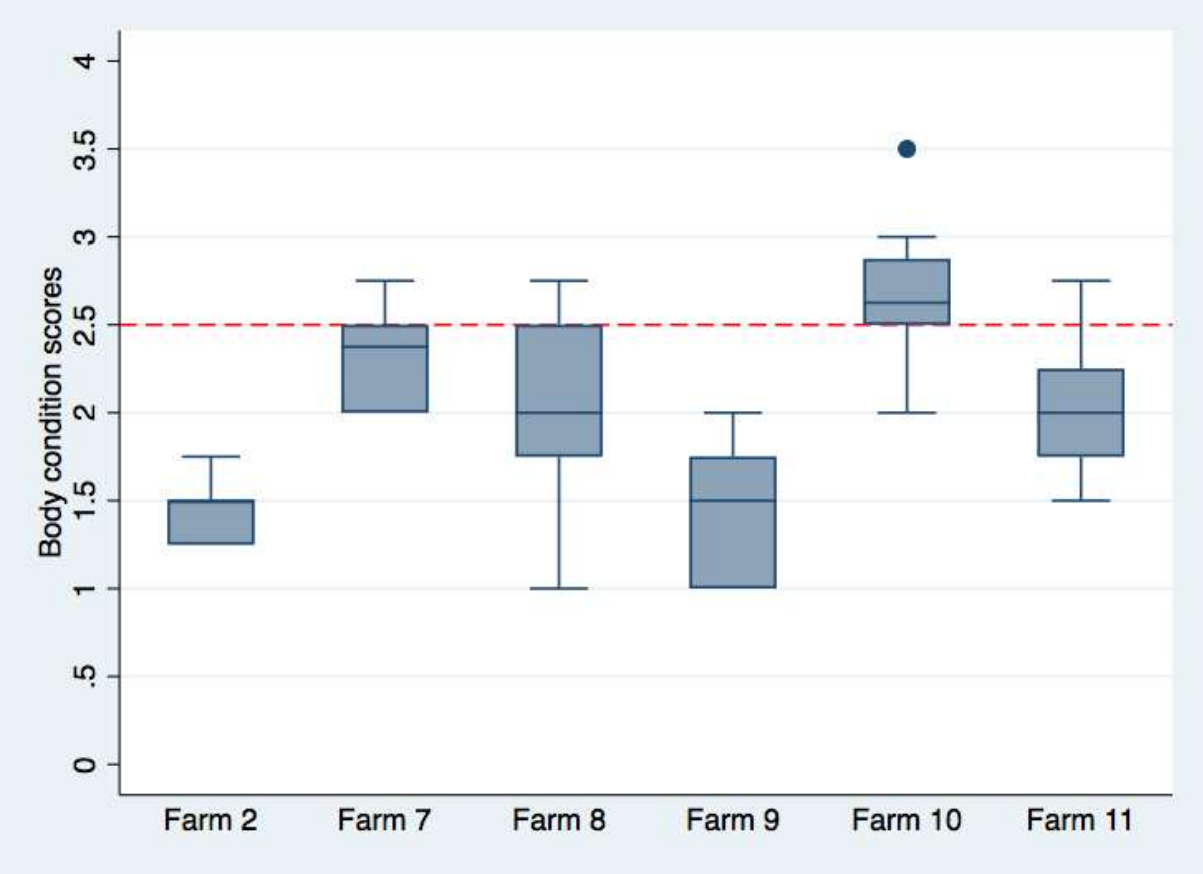


Figure 2: Box and whisker plots of body condition scores for ewes from upland/hill farms presented pre-tupping in Autumn 2018. The red dashed line reflects the target BCS for ewes for these farms.

Blood indicators of energy and protein status

Blood indicators of energy status include the ketone body β-hydroxybutyrate (BOHB), urea and non-esterified fatty acids (NEFA). Protein status can be assessed using urea, total blood protein and albumin. Taken together, these indicators can help interpret whether the ewes are in a stable state with regard to their energy and protein needs or whether there are imbalances in supply and demand.

Various factors can influence their interpretation for example the presence of some parasites e.g. *Fasciola hepatica*, and the presence of some disease processes e.g. Johnes disease (*Mycobacterium avium* spp. *paratuberculosis*).

For all the farms (Table 2) the mean BOHB concentrations were well within the normal range, indicating that a severe acute state of imbalance was not present. However, both the urea and NEFA concentrations indicated that the ewes may be under supplied with energy on all farms, except farm 2. An elevation in urea, combined with low albumin and high NEFA concentration can indicate insufficient energy necessary to incorporate the urea into microbial crude protein, resulting in it accumulating within the

blood. As such the elevations in urea seen were interpreted as a lack of energy, rather than an oversupply of protein. In addition, the elevations in NEFAs were likely to have resulted from the breakdown of fat through a chronic imbalance between energy needs and supply and were also interpreted as a lack of energy. Albumin was low on all farms, suggesting either a chronically inadequate supply of protein or concurrent protein loss. However, only farms 5 and 10 had *F. hepatica* eggs detected in pooled faecal samples, which could be contributing to protein loss; Johnes antibodies were not measured.

These factors, taken together with the low BCSs, indicated that ewes on all farms were either under supplied with energy and protein, or in some cases losing protein. In general, this was likely to be due to the lack of forage caused by the summer drought, however parasitism by *F. hepatica* may have compounded this and explain some of the low albumin concentrations in some ewes. The ewes sampled from each flock were taken from the productive ewe flock and no farm had a history of Johnes disease and so It was considered unlikely that the low albumin concentrations were associated with Johnes disease, although this could not be ruled out completely. Indeed, farm level prevalence of Johnes disease is unknown in Wales, although is likely to be underdiagnosed (11). All farms were advised to consider Johnes disease as a possibility in cases of chronic weight loss even when well supplied with food and to consider investigating ewes that die at postmortem or culled due to lack of body condition.

Specific advice was formulated for each farm to maximise intakes and increase body condition as much as possible prior to tupping. One major concern was that too rapid an increase in body condition so close to tupping could increase twinning rates for thinner ewes resulting in an increased risk of pregnancy toxaemia in late pregnancy for those ewes.

In the future, the farmers were advised to body condition score ewes at weaning and review the available forage at that point (12), together with the parasite forecasts available. Then, a meaningful plan could be formulated in order that ewes were in optimum condition for tupping. In years where similar droughts are experienced, lambs could be sold earlier as stores to prioritise grazing for ewes. Alternatively, some supplemental forage (and/or concentrate feed) may be needed on some farms in order to avoid sheep losing body condition or failing to gain sufficient body condition prior to tupping.

Farm ID	BOHB (mmol/l) mean (SD) [range] interp	Urea (mmol/l) mean (SD) [range] interp	NEFA (mmol/l) mean (SD) [range] interp	Albumin (g/l) mean (SD) [range] interp	Total protein (g/l) mean (SD) interp	Presence of *F. hepatica* eggs/*Paramphistome* spp. eggs
1	0.22 (0.05) [0.13-0.30] normal	7.64 (0.82) [6.30-8.50] **high**	0.47 (0.23) [0.28-1.04] **above normal**	22.47 (1.89) [18.70-25.00] **low**	70.0 (6.3) [61.80-80.80] normal	No – **Yes**
2	0.45 (0.14) [0.13-0.58] normal	6.31 (2.73) [0.06-8.40] normal	0.16 (0.02) [0.13-0.21] normal	21.34 (1.87) [19.20-24.60] **low**	67.8 (3.8) [62.00-72.40] normal	No – **Yes**
3	0.27 (0.04) [0.21-0.34] normal	10.34 (1.29) [7.10-11.40] **high**	0.56 (0.16) [0.33-0.79] **above normal**	21.59 (1.81) [18.40-24.10] **low**	70.6 (4.2) [65.50-79.90] normal	No – No
4	0.25 (0.04) [0.19-0.32] normal	5.75 (1.22) [4.00-7.55] normal	0.89 (0.21) [0.58-1.15] **above normal**	22.16 (0.47) [21.60-22.90] **low**	66.4 (5.0) [60.50-73.60] normal	No – No
5	0.39 (0.12) [0.25-0.61] normal	7.56 (1.97) [5.30-11.90] **high normal**	0.65 (0.29) [0.25-1.20] **above normal**	25.10 (2.27) [21.00-28.70] **low**	77.9 (6.7) [69.80-87.60] normal	**Yes** – No
6	0.32 (0.06) [0.26-0.41] normal	9.07 (1.11) [7.10-10.40] **high**	0.67 (0.25) [0.41-1.21] **above normal**	25.39 (2.30) [21.7028.70] **low**	86.0 (7.1) [69.50-94.80] **high**	No – No
7	0.25 (0.05) [0.17-0.32] normal	8.06 (2.13) [5.00-12.70] **high**	0.48 (0.19) [0.28-0.77] **above normal**	27.64 (2.78) [24.00-32.60] **low**	87.0 (5.9) [76.60-97.00] **high**	No – No
8	0.47 (0.16) [0.34-0.83] normal	8.36 (2.42) [4.20-11.10] **high**	0.56 (0.19) [0.32-0.81] **above normal**	27.38 (1.45) [25.50-30.10] **low**	83.4 (4.0) [79.40-92.20] **high**	No – No
9	0.37 (0.12) [0.23-0.62] normal	8.55 (1.26) [7.50-10.90] **high**	0.30 (0.07) [0.19-0.39] normal	25.05 (1.55) [23.60-28.60] **low**	77.3 (8.7) [63.90-90.20] normal	No – **Yes**
10	0.44 (0.11) [0.23-0.62] normal	6.93 (0.95) [5.50-8.80] normal	0.55 (0.23) [0.30-0.99] **above normal**	23.17 (2.19) [17.90-25.40] **low**	76.3 (7.0) 65.40-88.60] normal	**Yes** – No
11	0.38 (0.10) [0.24-0.58] normal	7.95 (1.91) [5.20-10.60] **high**	0.61 (0.22) [0.35-1.01] **above normal**	22.91 (1.13) [20.90-24.30] **low**	65.3 (4.2) [58.50-72.00] normal	No – **Yes**
12	0.39 (0.07) [0.28-0.46] normal	9.11 (0.56) [8.50-10.20] **high**	0.42 (0.21) [0.16-0.83] **above normal**	20.56 (2.51) [17.60-24.50] **low**	63.2 (5.6) [57.00-73.30] normal	No - No
Reference ranges	<0.8 mmol/l	2.8-7.1 mmol/l	<0.40 mmol/l	30-48 g/l	60-79 g/l	

Table 2: Blood indicators of energy and protein status for ewes sampled from the 12 farms pre-tupping in Autumn 2018, together with the results of faecal examination for *Fasciola hepatica* eggs and *Paramphistome* spp. eggs. Interpretations of the blood analytes are based on ranges supplied by the NUVetNA laboratory.

Parasitism

At the health examination, pooled faecal samples were obtained and analysed for the presence of adult *Fasciola hepatica* eggs and *Paramphistome* spp. eggs using standard sedimentation methods (13, 14), together with gastrointestinal nematode eggs, using a standard McMaster salt flotation technique (15). From these samples, two farms had *Fasciola hepatica* eggs detected (evidence of a patent fluke infection), three farms had *Paramphistome* spp. eggs detected (rumen fluke infection) and one farm had a high nematode faecal egg count. Using these data, together with detailed historical trends supplied by each farmer, and making use of the parasite forecast available from NADIS, individual parasite control advice was formulated.

Blood haematology data

A large amount of blood haematology data were produced from the blood samples collected. Summary data only are supplied (Table 3).

Nine flocks had evidence of some sheep with a mild anaemia. Those sheep below the threshold were only just below in most cases, with a few exceptions demonstrating a more marked anaemia, with two individuals with a haematocrit below 20%. Examination of the other red cell parameters indicated that in general these anaemias were non-regenerative. Unfortunately, no specific reason for this was found at either flock or individual level but could be reflective of the poor availability of grass with some sheep struggling more than others, or could reflect some underlying and undetected disease process in that individual. For example, some of the group were coughing when examined and one week later other groups of sheep on the farm developed coughing symptoms.

Whilst some flocks had small proportions of sheep with either elevated or reduced numbers of circulating white blood cells (which can crudely indicate evidence of a systemic infection), all flocks had sheep with one or other of the specific cell types elevated. In some cases, this could be correlated to the presence of clinical disease; for example, a number of sheep were noted to be coughing in flocks 3 and 10 at presentation. In addition, some individual sheep had infectious foot lesions present e.g. footrot, which could also explain some of the observed haematological changes e.g. elevations in monocytes and neutrophils for those affected individuals. Also, eosinophils may increase in response to parasitism and most of the flocks were carrying at least a small parasite burden. However, even flocks with no clinical evidence of disease showed proportions of sheep with elevation in one or other cell type and this was challenging to interpret.

Farm ID	N (%) sheep with low HCTs	N (%) sheep with elevated total WBCs	N (%) sheep with below normal total WBCs	N (%) sheep with elevated neutrophils	N (%) sheep with elevated lymphocytes	N (%) sheep with elevated monocytes	N (%) sheep with elevated eosinophils	N (%) sheep with elevated basophils
1	0/9 (0.0)	**1/9 (11.1)**	0/9 (0.0)	**2/11 (18.2)**	**2/11 (18.2)**	**10/11 (90.9)**	**6/11 (54.6)**	**4/9 (44.4)**
2	**4/8 (50.0)**	**1/8 (12.5)**	0/8 (0.0)	**2/9 (22.2)**	**1/9 (11.1)**	**5/9 (55.6)**	**7/9 (77.8)**	**4/7 (57.1)**
3	**3/8 (37.5)**	0/8 (0.0)	0/8 (0.0)	**5/13 (38.5)**	**5/13 (38.5)**	**12/13 (92.3)**	**6/13 (46.2)**	**5/10 (50.0)**
4	0/6 (0.0)	**1/6 (16.7)**	0/6 (0.0)	**5/9 (55.6)**	**3/9 (33.3)**	**8/9 (88.9)**	**3/9 (33.3)**	**3/7 (42.9)**
5	**1/7 (14.3)**	0/7 (0.0)	0/7 (0.0)	**3/10 (30.0)**	**3/10 (30.0)**	**9/10 (90.0)**	**8/10 (80.0)**	**5/7 (71.4)**
6	**1/8 (12.5)**	0/7 (0.0)	0/7 (0.0)	**4/11 (36.4)**	**4/11 (36.4)**	**10/11 (90.9)**	**7/11 (63.6)**	**5/11 (45.5)**
7	**2/8 (25.0)**	0/8 (0.0)	0/8 (0.0)	**2/10 (20.0)**	**2/10 (20.0)**	**10/10 (100)**	**3/10 (30.0)**	**2/7 (28.6)**
8	**1/8 (12.5)**	0/8 (0.0)	0/8 (0.0)	**2/10 (20.0)**	**2/10 (20.0)**	**10/10 (100)**	**2/10 (20.0)**	**2/10 (20.0)**
9	**2/8 (25.0)**	0/8 (0.0)	**2/8 (25.0)**	**2/10 (20.0)**	**2/10 (20.0)**	**6/10 (60.0)**	**4/10 (40.0)**	**4/7 (57.1)**
10	**4/10 (40.0)**	0/10 (0.0)	**1/10 (10.0)**	**3/13 (23.1)**	**3/13 (23.1)**	**6/13 (46.2)**	**3/13 (23.1)**	**3/7 (42.9)**
11	0/7 (0.0)	0/7 (0.0)	0/7 (0.0)	**3/10 (30.0)**	**3/10 (30.0)**	**7/10 (70.0)**	**3/10 (30.0)**	**3/6 (50.0)**
12	**1/7 (14.3)**	0/7 (0.0)	0/7 (0.0)	**2/8 (25.0)**	**1/8 (12.5)**	**6/8 (75.0)**	**1/8 (12.5)**	**2/8 (25.0)**
Reference ranges	27.0-42.0%	5.06-14.12x10^9/L		1.17-6.11x10^9/L	2.54-9.60x10^9/L	0.1-1.01x10^9/L	0.05-0.95x10^9/L	0.0-0.12x10^9/L

Table 3: Haematology data from the sheep sampled pre-tupping in Autumn 2018. Interpretations are based on ranges supplied by IDEXX and are suitable for the analyser used and for sheep.

Trace elements

Only one farm (Farm 3) had up-to-date information regarding the trace element status of the land, grass or the sheep; two other farms had employed some form of trace element testing of sheep in previous years. The remainder had never carried out any testing or investigation before. Varying trace element supplementation strategies were employed on the farms (Table 4). Most farmers reported that they were buying their trace element supplements based on what a neighbour was doing, as well as advice from a merchant selling trace element supplements.

Farm ID	Prior approach to trace element management in ewes	Trace elements supplied	Trace element status known
1	Mayo All Guard Ewe Bolus, given pre-tupping	Co I Se Zn	No
2	None	-	No
3	Bimeda COSEICURE Bolus given pre-tupping	Cu Co I Se	Yes
4	DM Mix (trace element mixture) given pre-tupping twice	Cu Co I Se	No
5	Rumevite SUPALyx bucket	Co I Se Zn Mn	Partially
6	None	-	No
7	Brinicomb Stockbooster Ewe Drench pre-tupping (Cu Co I Se Zn Mn); Animax Tracesure CuCoISe bolus at scanning.	Cu Co I Se Zn Mn	No
8	Animax Tracesure bolus CuCoSeI 6 weeks before lambing	Cu Co I Se	Partially
9	None	-	No
10	Crystalyx buckets pre-tupping; Se and Co drench at weaning (just ewes) as go up to mountain	Co I Se Zn Mn	No
11	Animax Tracesure bolus CuCoSeI 6 weeks before lambing	Cu Co I Se	No
12	Animax Tracesure CoISe bolus pre tupping	Co I Se	No

Table 4: Approach to trace element management in the ewes prior to commencement of the project.

Investigation for all flocks was carried out utilising forage (grass and conserved forage) samples, liver biopsies and blood samples taken from ewes pre-tupping. The samples were collected pre-tupping for two reasons: 1. in order to provide supplementation where needed prior to this critical period of production; 2. due to the fluctuating nature of trace elements within the sheep, sampling was carried out pre-tupping in order to ascertain the current as well as historic trace element status (where possible) in order to make an informed and evidence based supplementation choice.

From an analysis of the data the flocks were diagnosed as either likely or unlikely to benefit from supplementation (Table 5 and Table 6).

Farm ID	Liver tissue and blood results												Advice
	TISSUE Copper µmol/kg DM mean (SD) [range] *interpretation*	BLOOD Plasma copper µmol/l	BLOOD Caeruloplasmin mg/dl mean (SD) [range] *interpretation*	BLOOD Superoxide dismutase U/g Hb mean (SD) [range] *interpretation*	TISSUE Selenium µmol/kg DM mean (SD) [range] *interpretation*	BLOOD Plasma selenium µmol/l mean (SD) [range] *interpretation*	BLOOD Glutathione peroxidase U/ml PCV mean (SD) [range] *interpretation*	Status change: Glutathione peroxidase/ Plasma selenium	TISSUE Cobalt µmol/kg DM mean (SD) [range] *interpretation*	BLOOD Plasma cobalt µmol/l mean (SD) [range] *interpretation*	BLOOD Plasma zinc µmol/l mean (SD) [range] *interpretation*	TISSUE Manganese µmol/kg DM mean (SD) [range] *interpretation*	Black: no change; Red: change from previous practice
1	1228 (703.8) [467-2424] *below normal/normal*	10.89 (1.68) [8.7-12.8] *marginally low*	20.8 (5.5) [14.0-30.1] *normal*	2043 (371) [1602-2799] *normal*	4.75 (0.74) [3.78-6.11] *deficient/marginal deficient*	0.57 (0.12) [0.40-0.70] *sub-optimal*	77 (22) [46-123] *sub-optimal*	Down	1.88 (0.67) [0.95-2.82] *below normal/normal*	4.1 (0.8) [3.3-5.6] *marginally low*	11.1 (1.7) [7.9-13.2] *marginally low*	152.3 (39.4) [76.1-193.9] *normal*	Co Se Zn bolus pre-tupping
2	1195 (1073) [239-3206] *below normal/normal*	10.0 (1.7) [7.4-13.0] *marginally low*	21.5 (4.1) [15.8-29.3] *normal*	1930 (617) [853-2558] *marginally low/normal*	3.80 (0.80) [2.91-5.30] *deficient*	0.37 (0.10) [0.25-0.50] *sub-optimal*	47.9 (17.9) [26-82] *sub-optimal*	Down	2.33 (0.77) [1.75-3.99] *below normal/normal*	4.1 (0.6) [3.2-5.0] *marginally low*	8.9 (1.3) [6.4-10.2] *marginally low*	217.2 (26.9) [190.9-266.0] *normal*	**Co Se Zn bolus pre-tupping**
3	6075 (3052) [603-11085] *above normal/high*	9.8 (2.2) [7.9-14.6] *marginally low*	22.6 (6.0) [16.1-33.8] *normal*	2196 (211) [1771-2489] *normal*	6.97 (0.94) [5.39-8.82] *marginal deficient*	0.59 (0.12) [0.43-0.77] *sub-optimal*	156.3 (56.0) [57-217] *normal/marginally high*	Down	2.80 (1.31) [1.13-5.22] *normal*	4.7 (0.8) [3.4-6.0] *marginally low*	9.2 (1.9) [6.4-11.3] *marginally low*	246.4 (23.4) [210.5-281] *normal*	Se Co drench pre-tupping and scanning
4	314 (203) [108-685] *marginal deficient*	11.5 (4.4) [6.1-18.8] *marginally low*	21.4 (9.4) [12.6-38.4] *normal*	1394 (363) [902-1966] *low*	4.39 (1.48) [2.17-6.12] *deficient*	0.49 (0.17) [0.36-0.82] *sub-optimal*	32.3 (18.2) [10-52] *deficient/marginally low*	Up	3.63 (1.16) [2.05-5.33] *normal*	32.9 (21.0) [12.3-65.3] *normal/recent treatment?*	9.6 (2.5) [6.8-13.3] low/*marginally low*	125.5 (34.4) [72.6-186.4] *below normal/normal*	Cu Co Se drench pre-tupping and scanning
5	1230 (1618) [117-3736] deficient/below normal/normal	8.0 (3.0) [4.5-11.9] *low*	17.5 (9.1) [6.4-32.3] *low/normal*	1675 (395) [1188-2371] *low/marginally low*	4.99 (2.37) [2.45-7.77] *deficient/marginal deficient*	0.80 (0.31) [0.33-1.31] *sub-optimal/normal*	106.8 (52.9) [33-192] *normall*	Down	2.14 (1.13) [0.62-4.02] *below normal/normal*	4.4 (0.3) [4.1-4.9] *marginally low*	10.2 (0.9) [9.0-12.1] *marginally low*	153.5 (80.7) [53.3-277.2] below *normal/normal*	Cu Co Se Zn pre-tupping. A bolus would lead to less variation between sheep than buckets
6	536 (354) [83-1320] *marginal deficient*	9.0 (2.4) [6.5-12.7) *low*	23.2 (7.0) [15.5-35.4] *normal*	1754 (296) [1472-2220] *low/marginally low*	3.96 (0.52) [2.64-4.68] *deficient*	0.50 (0.09) [0.36-0.65] *sub-optimal*	60.0 (24.5) [31-98] *sub-optimal*	Down	3.20 (0.61) [2.35-4.51] *normal*	5.76 (1.55) [3.90-8.10] *marginally low/normal*	10.3 (1.5) [8.7-13.8] *low/marginally low*	183.9 (30.3) [144.1-254.5] *normal*	Cu Se Zn bolus pre-tupping

Table 5: Summary trace element findings and advice given pre-tupping 2018, farms 1-6. Interpretations of the analytes are based on ranges supplied by the NUVetNA laboratory. Graphical representations of these data are displayed in the appendix.

Farm ID	Liver tissue and blood results												Advice
	TISSUE Copper µmol/kg DM mean (SD) [range] *interpretation*	BLOOD Plasma copper µmol/l	BLOOD Caeruloplasmin mg/dl mean (SD) [range] *interpretation*	BLOOD Superoxide dismutase U/g Hb mean (SD) [range] *interpretation*	TISSUE Selenium µmol/kg DM mean (SD) [range] *interpretation*	BLOOD Plasma selenium µmol/l mean (SD) [range] *interpretation*	BLOOD Glutathione peroxidase U/ml PCV mean (SD) [range] *interpretation*	Status change: Glutathione peroxidase/ Plasma selenium	TISSUE Cobalt µmol/kg DM mean (SD) [range] *interpretation*	BLOOD Plasma cobalt µmol/l mean (SD) [range] *interpretation*	BLOOD Plasma zinc µmol/l mean (SD) [range] *interpretation*	TISSUE Manganese µmol/kg DM mean (SD) [range] *interpretation*	Black: no change; Red: change from previous practice
7 a	3405 (2489) [30-6038] *normal*	11.1 (2.1) [9.8-14.7] *marginally low*	24.9 (8.5) [19.2-39.6] *normal*	2136 (388) [1758-2706] *normal*	6.39 (3.39) [3.62-11.84] *deficient/marginally low*	0.90 (0.39) [0.34-1.23] *sub-optimal/normal*	217.0 (63.0) [161.0-296.0] *high*	Down	1.66 (0.91) [0.38-2.84] *below normal*	7.82 (3.40) [5.0-13.1] *normal*	8.5 (1.6) [6.3-10.4] *low*	90.6 (45.4) [24.3-136.9] *below normal*	Co Se Zn bolus pre-tupping/at scanning
7 b	2179 (3128) [83-7705] *normal*	14.6 (5.6) [10.4-24.4] *normal*	34.5 (11.8) [25.6-55.1] *normal*	1898 (159) [1653-2046] *marginally low/normal*	5.57 (2.56) [1.40-8.02] *deficient/marginally low*	0.64 (0.11) [0.53-0.79] *sub-optimal*	128.8 (31.4) [92.0-179.0] *normal*	Down	1.74 (1.02) [0.32-2.86] *below normal/normal*	5.22 (0.69) [4.20-6.00] *normal*	10.6 (1.5) [9.3-12.2] *low*	199.3 (57.0) [146.1-261.4) *normal*	Co Se Zn bolus pre-tupping/at scanning
8	1879 (1461) [257-4630] *marginal deficient/normal*	13.0 (3.4) [9.5-18.7] *marginally low/normal*	29.3 (3.2) [25.4-34.8] *normal*	1460 (383) [717-1940] *low*	5.21 (1.04) [4.15-7.32] *marginally deficient*	0.76 (0.36) [0.43-1.48] *sub-optimal/normal*	58.0 (28.0) [33.0-108.0] *sub-optimal*	Up	3.01 (0.89) [1.63-4.11] *below normal*	7.64 (0.94) [6.60-9.50] *normal*	11.8 (3.0) [8.5-16.8] *low/marginally low*	228.6 (60.4) [126.7-296.2] *normal*	Cu Co Se Zn bolus given pre-tupping
9	1271 (1290) [136-3938] *below normal*	9.2 (1.8) [5.9-11.5] *low/marginally low*	22.7 (6.2) [12.4-31.7] *normal*	1316 (629) [517-2208] *low*	9.20 (3.81) [2.47-13.81] *marginally low*	1.15 (0.61) [0.60-2.49] *normal*	216.3 (113.8) [66.0-368.0] *normal/high*	No change	2.62 (1.01) [0.60-3.83] *normal*	3.64 (0.52) [3.10-4.30] *marginally low*	8.4 (1.1) [6.7-9.9] *low*	180.6 (51.7) [65.2-239.8] *normal*	Cu Zn could be useful.
10	1930 (1594) [116-5990] *normal*	16.0 (5.1) [6.2-22.7] *normal*	35.8 (12.1) [12.2-50.2] *normal/acute phase*	2146 (277) [1769-2550] *normal*	12.93 (4.20) [7.31-20.81] *marginally low/normal*	1.83 (0.15) [1.51-2.07] *marginally high*	347.3 (63.3) [289-499] *high*	Down	2.71 (1.21) [0.58-4.50] *normal*	13.76 (11.05) [7.20-43.90] *normal*	10.5 (1.7) [8.1-13.3] *marginally low*	214.7 (87.4) [84.3-371.5] *normal*	No supplementation required
11	1440 (664) [499-2704] *normal*	12.9 (2.5) [10.6-17.8] *marginally low/normal*	26.8 (6.1) [16.7-35.3] *normal*	1823 (273) [1374-2348] *marginally low*	5.75 (1.89) [2.96-8.32] *marginally deficient*	0.59 (0.10) [0.41-0.76] *sub-optimal*	84.5 (25.5) [30-105] *normal*	Down	3.96 (1.45) [1.98-6.79] *normal*	12.70 (8.44) [5.20-31.10] *normal*	9.1 (1.5) [6.8-11.1] *marginally low*	149.4 (63.1) [34.6-238] *normal*	Cu Co Se Zn bolus given pre-tupping
12	3434 (1863) [1319-7357] *normal*	12.4 (2.5) [9.4-15.3] *marginally low*	25.5 (6.6) [18.4-38.2] *normal*	2065 (208) [1694-2419] *normal*	4.81 (1.31) [2.91-6.29] *deficient/marginally deficient*	0.49 (0.18) [0.25-0.73] *sub-optimal*	74.0 (28.1) [46.0-125.0] *sub-optimal*	Down	2.92 (0.56) [2.32-4.13] *normal*	5.31 (0.86) [4.40-7.10] *normal*	7.1 (0.7) [6.3-8.4] *low*	322.0 (198.3) [183.4-791.5] *above normal*	Se Co Zn bolus given pre-tupping

Table 6: Summary trace element findings and advice given pre-tupping 2018, farms 7-12. Interpretations of the analytes are based on ranges supplied by the NUVetNA laboratory. For Farm 7, 'a' denotes group 1 (BFL x Tex) and 'b' group 2 (Welsh). Graphical representations of these data are displayed in the appendix.

Interpretation	Copper (µmol/kg DM)	Manganese (µmol/kg DM)	Selenium (µmol/kg DM)	Cobalt (µmol/kg DM)
Deficient	<225		<4.5	
Marginally deficient	281-1124		6.8-11.3	
Below normal	1124-1405	<130		<2
Normal	1405-5619	130-286	11.3-67.8	2-5
Above normal	5619-8000	286-325	67.8-90.5	5-303
High	8000-14047	>325	90.5-452.3	
Toxic	>14047	???	>678.5	>303

Table 7: Ranges used in the interpretation of the liver tissue data as supplied by NUVetNA. All values are in µmol/kg DM assuming DM 280g/kg.

Interpretation	Plasma copper (µmol/l)	Caeruloplasmin (mg/dl)	Superoxide dismutase (U/g Hb)
Very low	<5.0		<1200
Low	5.0-9.4	<15.0	1200-1800
Marginally low	9.4-12.0		1800-2000
Normal	12.0-19.0	15.0-35.0	2000-2500
Marginally high	19.0-23.0		
High	>23.0	>35.0*	>2500
Too high			

Table 8: Ranges used in the interpretation of the copper blood data as supplied by NUVetNA. * Possibly acute phase

Interpretation	Plasma selenium (µmol/l)	Glutathione peroxidase (U/ml PCV)
Deficient	<0.22	<20.0
Marginally low	0.22-0.40	20.0-40.0
Sub-optimal	0.40-0.80	40.0-80.0
Optimal	0.80-1.50	80.0-180.0
Marginally high	1.50-2.00	180.0-210.0
High	>2.00	>210.0

Table 9: Ranges used in the interpretation of the selenium blood data as supplied by NUVetNA.

Interpretation	Plasma cobalt (µmol/l)	Plasma zinc (µmol/l)
Low	<3	<8
Marginally low	3-5	8-12
Normal	5-15	12-20
High	>15*	>20

Table 10: Ranges used in the interpretation of the cobalt and zinc blood data as supplied by NUVetNA. * Possibly recent treatment?

The farmers were free to choose whether to adopt the advice given or not, although most chose to do so. Two of the 12 farms were advised to make no change to their practice. For the other 10 farms, in order to optimise supplementation based on the available evidence, they were advised to either change the supplementation given, change its composition, change the timing it was given or change the way it was given i.e. from a bucket to a bolus.

Two farms chose not to follow this advice. Farm nine chose not to as there was concern from the farmer that whilst the ewes could benefit from copper and zinc, the expected scanning percentage was already too high for optimal and efficient production on this hill farm. An increase in the number of twins could result in an increase in subsequent lamb mortality as a result of ewes not being able to rear the extra lambs sufficiently well, together with an increase in workload to try and mitigate against this, as well as an extra burden on the affected ewes to recover for the following year. In addition, clinical swayback had not been diagnosed previously.

Farm 5 was advised to switch from supplementation using mineral buckets to a pre-tupping bolus supplying the same elements. This was based on the very wide variation in trace element concentrations between the sheep sampled, which was considered to possibly reflect the fact that some sheep utilise these buckets more than others, for reasons unknown. Using a bolus could mitigate this variation, however this farmer considered that whilst this approach may lead to a more uniform supplementation and potentially better health it would require more labour and therefore, he chose not to.

Forage analysis

Forage samples were obtained from each farm with sampling carried out based on the grazing structure of each farm and within the practical constraints of the project. Individual fields were grouped together for the purposes of grass sampling based on the geographical situation of the farm e.g. Sample 1: fields sampled one side of a river; Sample 2: fields sampled the other side of the river; Sample 3: baled silage. Where relevant, extensive mountain grazing was sampled in a transect from the summit down to the lowest point.

To obtain the mixed sample, grazed grass was cut using scissors, cutting the grass at approximately 2-5cm from the ground – the expected portion of the sward that would be grazed. Contamination with soil was avoided. Grass was collected using gloved hands and placed into clean unused polythene bags. Multiple cuts of grass were taken randomly throughout the grazed area. In order to obtain a random sample, the person sampling walked in a ‘W’ shape taking cut samples every 20 paces. When sampling conserved baled forage, forage was taken from deep within each bale and a minimum of three bales were included per sample. When sampling conserved clamp silage,

forage was taken from deep into the clamp and 3-5 gloved handfuls were taken per sample from random points across the clamp face.

The results for each farm (Table 11 and Table 12) were used to inform the advice given together with the blood and liver tissue samples from the sheep.

Major elements (Table 11)

Calcium (Ca) and phosphorus (P)

Calcium and phosphorus are the major inorganic components of animals and they have several key functions. Both are found in the skeleton; calcium is needed for many enzyme systems as well as the coagulation of blood, and phosphorus is needed for the production of some proteins, nucleic acids and phospholipids, as well as being important for energy metabolism(16). These two elements need considering together in that their relative abundance is as important as their overall abundance independently; generally the ratio of calcium to phosphorus should be within the range 1:1 to 2:1(16).

Calcium and phosphorus were abundant in virtually all the samples, being low in one sample only. Most of the samples had a Ca:P ratio within the recommended range although there were some exceptions. For flock 11, the calcium to phosphate ratio was low, although the overall abundance of calcium was high (Ca:P ratio 0.88:1). This could have resulted in a relative hypocalcaemia in heavily pregnant ewes, or those lactating, if not supplied with additional calcium. Conversely, the relatively high Ca:P ratio for the silage fed to flock 4 (2.89:1) was unlikely to cause any problems as the overall availability of phosphorus was adequate.

Potassium (K) and magnesium (Mg)

Potassium is important for osmotic regulation as well as maintaining the acid-base balance. It is generally present in high concentrations in grass and was at high concentrations in all the samples tested.

Magnesium is found in the skeleton but is also essential in many enzyme systems. The magnesium concentration was high in all the forage samples except two hay samples, so its overall availability was likely to be sufficient. However, it is reported by some workers that potassium can inhibit the absorption of magnesium by inhibiting the active transport systems within the rumen wall (16) and this was considered possible for most of these flocks despite the high magnesium concentrations for the majority of the samples tested. Indeed, most flocks had reported hypomagnesaemia (staggers) in previous years and this risk was reiterated.

Sulphur (S)

Sulphur is necessary for the synthesis of structural proteins as well as other functions. The forage sulphur concentration was high in nearly all the forage samples obtained, most notably in the samples from the more productive pastures compared to those from mountain samples. The high concentration observed in most of the samples is likely to be due to fertiliser application containing sulphur, hence the observed relationship with improved ground. Whilst a sulphur deficiency was unlikely for these flocks, the elevated sulphur could interact with molybdenum and/or iron impacting on the availability of copper to the sheep (16, 17).

Sodium (Na)

Sodium is important in maintaining hydration status as well as maintaining the acid-base balance in the body. In all the forage samples sodium was at optimal or high levels and therefore a deficiency was highly unlikely. The high concentrations were unlikely to cause any problems, provided animals were given sufficient free access to fresh water.

Farm ID	Sample	Ca g/kg DM	Ca status	P g/kg DM	P status	Ca:P	K g/kg DM	K status	Mg g/kg DM	Mg status	S g/kg DM	S status	Na g/kg DM	Na status
1	Grass	6.95	High	3.97	High	1.75	19.88	High	2.44	High	2.66	High	4.09	High
	Grass	4.66	High	3.82	High	1.22	25.69	High	2.04	High	1.91	High	1.30	High
	Silage (round bale)	5.10	High	3.30	High	1.55	34.64	High	1.52	High	1.93	High	0.52	Optimal
2	Grass (mountain)	4.83	High	4.79	High	1.01	27.19	High	2.27	High	2.42	High	0.62	Optimal
	Grass (lowlands)	5.96	High	4.47	High	1.33	27.66	High	1.76	High	2.69	High	0.73	Optimal
	Hay	4.06	High	2.40	High	1.69	22.06	High	1.09	Optimal	1.16	Low	1.65	High
3	Silage (round bale)	4.22	High	2.78	High	1.52	18.92	High	2.13	High	2.61	High	8.24	High
	Hay	6.59	High	2.22	High	2.97	15.48	High	1.9	High	2.02	High	7.46	High
4	Grass	6.63	High	5.40	High	1.23	37.69	High	2.81	High	3.73	High	1.16	High
	Silage (round bale)	5.21	High	1.80	Optimal	2.89	11.47	High	1.71	High	1.65	High[††]	5.24	High
5	Grass	5.92	High	4.81	High	1.23	40.20	High	2.24	High	3.9	High	1.15	High
	Hay	3.64	High	2.62	High	1.39	23.13	High	1.24	Optimal	1.05	Low	0.47	Low
6	Grass	6.18	High	4.04	High	1.53	26.45	High	2.25	High	2.8	High	3.91	High
	Grass	4.73	High	4.08	High	1.16	30.55	High	2.11	High	3.3	High	2.03	High
	Silage (clamp)	6.42	High	2.81	High	2.28	21.97	High	1.86	High	2.15	High	2.16	High
8	Grass	6.07	High	4.40	High	1.38	25.84	High	2.28	High	3.75	High	3.17	High
	Silage (round bale)	5.81	High	2.91	High	2.00	19.37	High	2.29	High	2.07	High	4.03	High
9	Grass	5.03	High	2.61	High	1.93	19.22	High	3.48	High	3.98	High	4.13	High
	Grass	4.28	High	2.32	High	1.84	17.51	High	2.8	High	2	High	1.69	High
	Grass	5.68	High	4.22	High	1.35	22.84	High	3.3	High	2.72	High	3.05	High
	Grass	5.50	High	3.30	High	1.67	20.51	High	2.82	High	2.57	High	2.18	High
10	Grass (mountain)	1.77	Low	1.16	Low	1.53	7.90	High	1.28	High	3.16	High	0.47	Low
	Grass (lowland)	4.26	High	3.85	High	1.11	30.83	High	2.75	High	1.41	Low	3.41	High
	Silage (round bale)	8.16	High	3.00	High	2.72	13.11	High	3.82	High	2.43	High	6.32	High
11	Grass	4.07	High	4.61	High	0.88	37.58	High	2.55	High	3.8	High	1.93	High
	Silage (round bale)	6.26	High	2.72	High	2.30	12.19	High	2.64	High	2.65	High	3.65	High
12	Grass	7.09	High	3.42	High	2.07	33.07	High	2.69	High	3.06	High	2.56	High
	Haylage	3.41	High	2.19	High	1.56	18.22	High	1.68	High	1.55	Low	2.62	High

Table 11: Forage analysis data for grass and conserved forage samples taken from each farm; interpretations are based on ranges provided by NUVetNA laboratories; based on the NRC requirements. [†]Within normal range but borderline low [††]Within normal range but borderline high

Trace elements (Table 12)

Iron (Fe)

Iron is important in the formation of red blood cells and a deficiency can result in a primary anaemia (16). The forage iron concentration was high in nearly all the forage samples obtained. Anaemia was not an issue in any of the sheep samples and it is unlikely in ewes with sufficient access to forage in the UK, although is possible in milk fed lambs. High iron concentrations together with sulphur can lead to reduced absorption of copper and can compound any absolute copper deficiencies or sulphur/molybdenum/copper interactions (17). The iron concentration of forage can increase with waterlogging and compaction so advice was given to consider where drainage improvements could be made if practical.

Zinc (Zn)

Zinc is important in enzyme systems, cell replication/metabolism, the formation of skin, horn and wool and is important in the production of immunoglobulins (18, 19). The concentration of zinc was variable between farms and between samples from the same farm. Some of the animal samples also showed low zinc concentrations (Table 5; Table 6). Advice was given to supplement with zinc where forage samples were shown to be unlikely to be able to support the animal and where blood analyses showed low concentrations.

Copper (Cu)

Copper is important for many reasons: 1) it is important in the formation of haemoglobin utilised by the protein caeruloplasmin enabling the release of iron from cells into the plasma; 2) it is necessary for iron absorption; 3) it occurs in proteins involved in oxygen metabolism; 4) it is necessary in some enzyme systems; 5) it has been shown to reduce the susceptibility of lambs to infection. In some areas, 'swayback' a congenital deficiency of copper with neurological effects is observed, particularly on pastures of low copper content (2-4mg/kg DM). Different breeds of sheep may be more or less affected due to their differing variability in absorption, for example the Texel breed may retain approximately twice as much copper as that retained by Blackface sheep (16). Copper absorption may also be affected by interactions with molybdenum and sulphur. Significant amounts of molybdenum react with sulphide, produced by ruminal microorganisms to form thiomolybdate, which then reacts with copper to produce copper thiomolybdate. This is insoluble and is then excreted from the body unabsorbed thus reducing the copper available to the sheep (16). Significant amounts of iron may also serve to inhibit copper absorption.

The measured copper concentration, assessed in isolation, was optimal for the majority of the samples, low in one sample and high in four. However, whilst

none of the pastures had particularly high molybdenum levels (in the region of 20-100mg/kg DM) some were above what could be considered ‘normal’ (0.5-3.0mg/kg DM) and all but one had high levels of sulphur present. As such, some of the observed copper deficiencies seen, for example for sheep from flock 4 (Table 5, Table 15 and Table 16), could be related to the relatively high molybdenum concentration of the grass, interacting with the high sulphur content and also potentially compounded by the high iron content too. The administration of a copper containing bolus in spring prior to returning to the grass pasture served to address this deficiency (Table 19).

Selenium (Se)

Selenium is important in the functioning of the immune system (19), in the production of thyroid hormones (alongside iodine), in the development of muscle, and importantly in the enzyme glutathione peroxidase which serves to catalyse the removal of hydrogen peroxide and therefore protect cells from oxidative damage (18). Many of the samples demonstrated low or borderline low concentrations of selenium. Advice was given to supplement with selenium prior to tupping and throughout pregnancy where forage samples had low/borderline low concentrations of selenium and where blood and tissue samples demonstrated sheep were under supplied.

Cobalt (Co)

Cobalt is required by microorganisms in the rumen to synthesize vitamin B12, which is essential for energy metabolism and in the production of red blood cells. Lambs have a much greater requirement for cobalt than ewes due to their need to grow and therefore deficiencies may be seen more commonly in lambs. In this project, the cobalt concentration of the forage samples varied widely, within and between farms. Cobalt concentration can vary within the sward quite markedly over the year and the amount available to sheep can vary with the amount of soil consumed (20). Advice was given to supplement with cobalt prior to tupping and throughout pregnancy where forage samples had low/borderline low concentrations of cobalt and where blood and tissue samples demonstrated sheep were under supplied. It might be expected that cobalt intakes would increase over the pregnancy period, however as repeated forage samples were not possible within this project and given the high toxic threshold for cobalt, supplementation was given where there was evidence some prevention may be beneficial.

Manganese (Mn)

Manganese is important as an activator in many enzyme systems, including those required in the formation of bone and may also have effects on fertility (16). All the forage samples had high concentrations of manganese and most of the sheep samples demonstrated adequate levels of manganese. In one

flock (flock 7) supplementation was recommended for one group of sheep, although it was not able to be carried out at the time due to practical constraints.

Farm ID	Sample	Fe mg/kg DM	Fe status	Zn mg/kg DM	Zn status	Cu mg/kg DM	Cu status	Mo mg/kg DM	Mo status	Se mg/kg DM	Se status	Co mg/kg DM	Co status	Mn mg/kg DM	Mn status
1	Grass	84	High	25.49	Low	6.00	Optimal	1.60	Normal	0.021	Low	0.034	Low	84.7	High
	Grass	395	High	35.19	Optimal	5.96	Optimal	1.68	Normal	0.050	Optimal	0.163	Optimal	121.5	High
	Silage (round bale)	190	High	23.16	Low	3.45	Low	1.16	Normal	0.015	Low	0.096	Low	68.9	High
2	Grass (mountain)	293	High	31.94	Optimal	6.76	Optimal	3.52	High	0.043	Low†	0.096	Low	178.6	High
	Grass (lowlands)	253	High	36.64	Optimal	6.14	Optimal	6.82	High	0.027	Low	0.126	Optimal	83.1	High
	Hay	63	High	24.01	Low	4.16	Optimal†	1.85	Normal	0.039	Low	0.045	Low	91.5	High
3	Silage (round bale)	99	High	33.32	Optimal	23.29	High	0.39	Low	0.082	Optimal	0.096	Low	384.9	High
	Hay	257	High	23.23	Low	6.56	Optimal	0.68	Normal	0.038	Low	0.077	Low	235.8	High
4	Grass	782	High	34.19	Optimal	10.54	High	9.55	High	0.047	Low†	0.291	High	41.7	High
	Silage (round bale)	398	High	32.4	Optimal	7.61	Optimal	0.82	Normal	0.024	Low	0.101	Low†	137.9	High
5	Grass	180	High	31.84	Optimal	10.39	High	4.91	High	0.042	Low†	0.068	Low	43.3	High
	Hay	33	Optimal	14.83	Low	4.64	Optimal	3.00	Normal	0.041	Low†	0.018	Low	45.0	High
6	Grass	897	High	71.35	High	10.89	High	2.15	Normal	0.109	Optimal	0.272	High	233.1	High
	Grass	1067	High	45.76	High	9.65	Optimal	3.89	High	0.087	Optimal	0.367	High	115.4	High
	Silage (clamp)	333	High	27.53	Optimal	7.72	Optimal	1.62	Normal	0.046	Low†	0.146	Optimal	130.2	High
8	Grass	808	High	39.63	Optimal	9.46	Optimal	1.13	Normal	0.067	Optimal	0.358	High	312.7	High
	Silage (round bale)	251	High	30.1	Optimal	5.88	Optimal	0.30	Low	0.040	Low†	0.104	Low†	374.2	High
9	Grass	108	High	37.59	Optimal	9.25	Optimal	4.18	High	0.045	Low†	0.192	Optimal	915.1	High
	Grass	90	High	43.5	Optimal	6.81	Optimal	0.65	Normal	0.047	Low†	0.063	Low	645.0	High
	Grass	102	High	34.33	Optimal	7.61	Optimal	1.71	Normal	0.084	Optimal	0.043	Low	351.4	High
	Grass	139	High	42.94	Optimal	7.10	Optimal	1.85	Normal	0.151	Optimal	0.074	Low	630.8	High
10	Grass (lowland)	211	High	24.1	Low	7.02	Optimal	1.35	Normal	0.065	Optimal	0.119	Optimal	74.5	High
	Grass (mountain)	105	High	35.9	Optimal	4.72	Optimal	0.73	Normal	0.086	Optimal	0.151	Optimal	556.8	High
	Silage (round bale)	630	High	42.33	Optimal	8.29	Optimal	0.33	Low	0.061	Optimal	0.298	High	286.0	High
11	Grass	139	High	35.5	Optimal	8.68	Optimal	1.70	Normal	0.056	Low†	0.110	Optimal	123.9	High
	Silage (round bale)	68	High	31.63	Optimal	7.92	Optimal	0.90	Normal	0.016	Low	0.097	Low	168.9	High
12	Grass	109	High	31.78	Optimal	8.33	Optimal	2.11	Normal	0.033	Low	0.034	Low	116.5	High
	Haylage	60	High	19.08	Low	4.13	Optimal†	0.89	Normal	0.017	Low	0.025	Low	146.3	High

Table 12: Forage analysis data for grass and conserved forage samples taken from each farm; interpretations are based on ranges provided by NUVetNA laboratories; based on the NRC requirements. †Within normal range but borderline low ††Within normal range but borderline high

Scanning results 2018/2019 and 2019/2020

Scanning results were reported by most of the farmers. Farm 12 did not scan and for farm 1 and farm 5 data were unavailable for 2019/2020 (Table 13). In 2018/2019 only three farms achieved scanning results close to the targets set by each farmer. The specific reasons for this are likely to be multifactorial and complex and are discussed later. In 2019/2020 scanning results were improved for five flocks; two flocks had dropped out of the project; two flocks had increased scanning percentages over the desired target, and one was stable but still above target (flock 9).

Farm ID	Target scanning percentage (%)	Scanning results 2018/2019	Scanning results 2019/2020	Comments regarding change between years
1	180	**160**	-	Changed enterprise
2	150	148	**142**	**Worse**, however had problems with fluke and scab; had *T. gondii* 2018/2019 - didn't vaccinate older ewes; Border disease identified 2019/2020.
3	180	**132**	**150**	**Improved**
4	180	**127**	**149**	**Improved,** didn't vaccinate against *T. gondii* despite empty ewes with high antibody titres
5	200	**180**	-	Dropped out of project
6	175	**160**	**165**	**Improved**
7	180; 150	**130; 130**	182; 149	**Improved,** met target
8	130	**122**	**125**	**Similar**
9	120	**160**	**160**	**Stable**, too many twins for this system
10	120	120	131	**Increased,** stable for this system
11	160	158	**170**	**Increased**, too many twins
12	150	-	-	Did not scan; data unavailable

Table 13: Scanning results for each flock for 2018/2019 and 2019/2020. Red coloration indicates a value below the target set; blue coloration a value above the target set; black colouration at or close to the target set.

Objective 2 – monitoring and evaluation

a) *Determine the energy and mineral status of pregnant breeding ewes in the last third of pregnancy prior to lambing.*
b) *Determine the energy and trace element status of a sample of breeding ewes post lambing.*

a) Pre-lambing monitoring and nutritional advice

Following on from the pre-tupping series of investigations, flocks were re-examined and re-sampled to evaluate the success of the advice given and to 'fine tune' the energy and protein delivery pre-lambing. Sheep were examined 3-4 weeks before lambing in order to make any changes needed before lambing.

Blood indicators of energy and protein status (Table 14) were utilised together with information regarding the current feeding of the ewes and their body condition to formulate adjustments to feeding plans as lambing approached and during lambing. Feed adjustments were made tailored to the estimated mean weight of the ewes and to the number of foetuses ewes were expected to be carrying and adjusted to the number of weeks prior to lambing with projections provided forwards of this.

Two flocks (numbers 8 and 11) were noted to be at high risk of pregnancy toxaemia with BOHB, urea and NEFA concentrations being elevated in a large proportion of the sample ewes. The majority of flocks appeared to be still dealing with the consequences of having ewes below target BCSs pre-tupping, with elevations noted in urea and NEFA concentrations particularly, as well as low albumin concentrations.

With regard to trace elements there was variation in the success of the management advice given (Table 15). For some flocks, trace elements appeared to be relatively well managed within or close to optimal or normal ranges e.g. flocks 3, 7, 8, 9 and 12. However, overall, further adjustments were likely to be necessary in future years based on these monitoring results. In particular the element zinc was low in several flocks and copper appeared low in others (see later). It had been expected for several farms that given the good concentrations of zinc in the available forage that the ewes would have been able to utilise that effectively, however it may have been the case that due to their increased nutritional demands resulting from the lack of forage over the summer this was still insufficient and further supplementation may have been beneficial.

Farm ID	BOHB (mmol/l) mean (SD) [range] interp	Urea (mmol/l) mean (SD) [range] interp	NEFA (mmol/l) mean (SD) [range] interp	Albumin (g/l) mean (SD) [range] interp	Total protein (g/l) mean (SD) [range] interp
1	0.66 (0.21) [0.46-1.17] normal	9.25 (1.64) [6.00-13.00] **high**	0.91 (0.50) [0.13-1.96] **above normal**	28.52 (2.24) [24.80-32.10] **low**	82.9 (5.2) [72.9-91.9] **high**/normal
2	0.56 (0.21) [0.30-1.08] normal	3.87 (1.26) [1.80-5.80] normal	0.42 (0.37) [0.04-1.20] **above normal**	29.38 (1.32) [27.00-31.80] **low**	82.1 (4.9) [75.3-91.5] **high**/normal
3	0.64 (0.12) [0.50-0.82] normal	8.52 (1.47) [5.60-10.30] **high**	0.64 (0.36) [0.16-1.13] **above normal**	25.02 (2.96) [19.50-28.30] **low**	79.6 (5.8) [71.7-90.3] **high**/normal
4	0.71 (0.15) [0.53-1.01] normal	9.41 (2.78) [5.60-13.0] **high**	1.17 (0.32) [0.74-1.72] **above normal**	27.01 (2.00) [23.00-29.90] **low**	84.3 (7.2) [75.8-99.6] **high**/normal
5	0.77 (0.23) [0.39-1.30] normal/**high**	12.93 (2.59) [9.10-18.10] **high**	1.11 (0.51) [0.31-2.15] **above normal**	29.31 (2.92) [23.50-33.40] **low**	82.3 (5.3) [76.4-94.8] **high**/normal
6	0.57 (0.13) [0.38-0.84] normal	7.19 (1.92) [5.60-12.00] normal/**high**	0.63 (0.42) [0.12-1.57] **above normal**	30.30 (1.38) [27.20-31.90] **low**/normal	81.4 (5.2) [75.2-88.0] **high**/normal
7	0.66 (0.27) [0.32-1.20] normal	10.29 (1.93) [4.70-14.00] **high**	0.73 (0.58) [0.03-2.33] **above normal**	28.23 (2.29) [23.80-32.80] **low**	81.2 (5.2) [71.2-90.1] **high**/normal
8	0.92 (0.42) [0.54-2.03] **high**	13.15 (2.09) [9.90-15.70] **high**	1.07 (0.38) [0.51-1.83] **above normal**	29.75 (0.94) [28.40-31.40] **low**	82.4 (7.0) [70.3-95.8] **high**/normal
9	0.56 (0.14) [0.38-0.87] normal	8.46 (1.55) [6.70-12.10] **high**	0.29 (0.18) [0.15-0.76] normal	30.71 (1.56) [28.50-33.30] **low**/normal	77.2 (2.7) [73.5-80.2] normal
10	0.84 (0.26) [0.55-1.38] normal/**high**	7.21 (1.36) [4.30-8.60] normal	0.66 (0.43) [0.19-1.42] **above normal**	28.79 (2.22) [24.20-31.20] **low**	81.5 (6.7) [74.5-93.7] **high**/normal
11	0.98 (0.36) [0.63-0.186] **high**	8.03 (2.42) [5.30-13.40] **high**	1.78 (0.55) [1.06-2.74] **above normal**	31.53 (2.39) [27.70-34.80] **low**/normal	81.4 (4.6) [72.9-90.4] **high**/normal
12	0.72 (0.26) [0.40-1.16] normal	6.59 (3.16) [3.20-12.30] normal	0.88 (0.34) [0.33-1.64] **above normal**	28.92 (1.84) [25.50-31.00] **low**	85.0 (7.6) [73.9-98.4] **high**/normal
Reference ranges	<0.8 mmol/l	2.8-7.1 mmol/l	<0.40 mmol/l	30-48g/l	60-79 g/l

Table 14: Blood indicators of energy and protein status for ewes sampled from the 12 farms 3-4 weeks before lambing in Spring 2019. Interpretations of the blood analytes are based on ranges supplied by the NUVetNA laboratory.

Farm ID	Blood results							
	BLOOD Plasma copper µmol/l mean (SD) [range] *interpretation*	BLOOD Serum Caeruloplasmin mg/dl mean (SD) [range] *interpretation*	BLOOD Superoxide dismutase U/g Hb mean (SD) [range] *interpretation*	BLOOD Plasma selenium µmol/l mean (SD) [range] *interpretation*	BLOOD Glutathione peroxidase U/ml PCV mean (SD) [range] *interpretation*	Status change: Glutathione peroxidase/Plasma selenium	BLOOD Plasma cobalt µmol/l mean (SD) [range] *interpretation*	BLOOD Plasma zinc µmol/l mean (SD) [range] *interpretation*
1	7.17 (4.49) [2.2-16.8] *low*	9* (11.7) [<2-36.5] *low*	2384 (457) [1662-3264] *normal*	0.70 (0.17) [0.46-0.96] *sub-optimal*	105 (27) [59-168] *optimal*	Down	12.1 (5.9) [5.3-26.7] *normal*	9.3 (1.2) [7.3-11.5] *low/marginally low*
2	13.6 (3.3) [7.0-18.6] *marginally low/normal*	23.4 (7.2) [9.6-34.9] *normal*	1529 (437) [800-2292] *low/marginally low*	1.60 (0.36) [0.74-1.97] *normal/marginally high*	164 (30) [109-201] *optimal/marginally high*	No change/Down	45.2 (30.5) [7.9-121.7] *high/recent treatment*	7.6 (1.4) [5.5-9.6] *low*
3	12.9 (2.3) [11.0-18.7] *marginally low*	25.3 (6.2) [18.1-39.5] *normal*	2207 (303) [1607-2541] *normal*	0.72 (0.23) [0.37-1.20] *sub-optimal*	138 (38) [88-203] *optimal*	Down	24.2 (13.9) [12.2-51.4] *high/recent treatment*	7.3 (0.7) [6.1-8.9] *low*
4	5.6 (2.3) [1.8-9.5] *very low/low*	8.6 (6.2) [<2-19.6] *low*	1664 (353) [958-2277] *low/marginally low*	0.40 (0.12) [0.21-0.63] *marginally deficient/sub-optimal*	75 (36) [27-141] *sub-optimal*	Down	12.8 (4.9) [6.8-24.4] *normal*	7.9 (0.7) [6.5-9.0] *low*
5	7.5 (4.1) [1.7-15.2] *low*	12.6* (9.4) [<2-29.6] *low*	1539 (390) [982-2269] *low*	1.28 (0.57) [0.39-2.18] *normal (but wide range)*	138 (68) [39-254] *normal (but wide range)*	No change (but wide range)	18.9 (7.0) [12.4-32.1] *recent treatment?*	9.4 (1.6) [6.9-12.3] *marginally low*
6	6.3 (3.8) [1.9-13.0] *low*	13.8 (9.6) [4.6-31.6] *low/normal*	1666 (276) [1287-2203] *low*	0.82 (0.38) [0.39-1.64] *normal (but wide range)*	93 (48) [38-194] *normal (but wide range)*	No change (but wide range)	12.7 (3.4) [8.0-17.4] *normal*	12.6 (1.9) [8.3-15.0] *marginally low*
7 a	12.9 (2.2) [10.8-17.6] *marginally low*	21.7 (3.8) [16.6-30.8] *normal*	2052 (268) [1396-2362] *marginally low/normal*	1.82 (0.23) [1.34-2.22] *marginally high/high*	235 (72) [144-374] *high*	Down	107.8 (36.3) [47.8-179.2] *recent treatment?*	8.0 (1.0) [6.1-10.0] *low*
7 b	13.4 (5.3) [9.7-27.9] *marginally low*	24.1 (7.5) [15.6-42.4] *normal*	1935 (338) [1175-2312] *marginally low*	1.80 (0.25) [1.32-2.20] *marginally high*	282 (75) [188-418] *high*	Down	57.8 (44.8) [11.3-140.8] *recent treatment?*	9.5 (1.0) [8.0-11.6] *low*
8	12.9 (1.4) [10.3-14.4] *marginally low*	21.4 (5.3) [9.4-27.1] *normal*	1894 (496) [893-2405] *marginally low*	1.68 (0.37) [1.09-2.26] *marginally high*	245 (69) [142-352] *high*	Down	63.1 (25.8) [21.4-100.9] *recent treatment?*	8.8 (0.8) [7.5-10.1] *low*
9	13.2 (0.8) [11.7-14.3] *marginally low*	29.2 (2.8) [24.9-33.6] *normal*	2208 (520) [1238-2719] *normal (but wide range)*	1.97 (0.18) [1.71-2.20] *marginally high/high*	247 (82) [139-343] *high*	Down	80.4 (35.6) [40.1-140.5] *recent treatment?*	11.0 (1.0) [8.5-12.0] *low*
10	11.6 (2.0) [7.0-13.8] *low*	21.5 (5.0) [10.6-28.6] *normal*	1864 (246) [1418-2209] *marginally low*	1.73 (0.23) [1.33-2.13] *marginally high*	373 (78) [262-525] *high*	Down	63.6 (35.8) [21.1-140.1] *recent treatment?*	8.6 (1.1) [6.9-11.1] *low*
11	13.1 (5.4) [2.1-23.4] *marginally low*	20.8 (11.0) [<2-33.2] *normal*	1492 (423) [858-1926] *low*	1.74 (0.26) [1.43-2.16] *marginally high*	226 (53) [137-309] *high*	No change/Down	56.6 (46.5) [15.0-170.2] *recent treatment?*	9.8 (1.3) [7.1-11.8] *low*
12	14.2 (7.8) [5.7-34.6] *marginally low*	27.7 (7.3) [16.5-37.3] *normal*	2200 (248) [1866-2562] *normal*	1.23 (0.19) [0.93-1.56] *normal*	261 (83) [121-365] *high*	Down	15.0 (6.4) [7.2-30.5] *normal*	12.0 (2.2) [8.7-15.9] *marginally low*

Table 15: Summary trace element findings for ewes sampled from the 12 farms 3-4 weeks before lambing in Spring 2019. Interpretations of the blood analytes are based on ranges supplied by the NUVetNA laboratory. *This number may be inaccurate as some of the values were <2 mg/dl and therefore unable to be computed accurately. For Farm 7, 'a' denotes group 1 (BFL x Tex) and 'b' group 2 (Welsh). Graphical representations of these data are displayed in the appendix.

Some of the ewes from flocks 1, 4, 5 and 6 had very low plasma copper concentrations (<5 µmol/l), very low caeruloplasmin concentrations (<2 mg/dl) and very low superoxide dismutase concentrations (<1200 U/g Hb). Concern was raised with these farmers about the very real possibility of swayback being observed in the lambs as a result. As lambing had commenced the opportunity for any intervention was limited, but very close monitoring was carried out and no clinical cases were observed. For flocks 1 and 4, liver tissue samples (n=8) were taken from new-born lambs that died from natural causes (e.g. dystocia, smothering, starvation etc.) and trace element analysis was carried out on these tissue samples (Table 16). This information was retained and carried through to the next phase in summer/autumn 2019.

With regard to flock 4, given the confirmed deficiency in the lambs and the understanding that the sheep would be grazing pasture with a relatively high sulphur and molybdenum content for approximately 9-10 months, the decision was taken to supplement the ewes with copper (using a bolus), alongside selenium and cobalt, as they left the shed and went back to pasture.

Farm ID	Lamb liver tissue results			
	Copper µmol/kg DM mean (SD) [range] *interpretation*	Selenium µmol/kg DM mean (SD) [range] *interpretation*	Cobalt µmol/kg DM mean (SD) [range] *interpretation*	Manganese µmol/kg DM mean (SD) [range] *interpretation*
1	1971 (2221) [111-5091] *normal (wide range from deficient to high normal)*	12.48 (3.26) [6.89-18.38] *marginally low/normal*	2.35 (1.31) [0.90-5.03] *below normal/normal*	258.3 (112.5) [154.1-445.9] *normal/high*
4	355 (412) [79-1223] *deficient/marginally low*	11.95 (5.60) [5.30-22.20] *normal/marginally low*	1.93 (0.73) [1.23-3.09] *below normal/normal*	282.3 (155.6) [98.4-623.0] *normal (wide range)*

Table 16: Summary trace element findings for lambs, sampled from 2 farms (farms 1 and 4) during lambing in Spring 2019. Interpretations of the analytes are based on ranges supplied by the NuVETNA laboratory.

b) Investigations, planning and monitoring post-weaning/pre-tupping 2019

General health 2019

As in 2018, each farmer presented a random selection of 20 ewes from the flock for inspection. These were body condition scored and a general assessment of their health was made. In general, these ewes were condition scored within four weeks post weaning, which was earlier than in 2018. The BCSs for the ewes had for some farms improved compared to 2018 but was still considerably below a pre-tupping target for five farms (10) (Figure 3). However, given the increased amount of time available before tupping there was a greater opportunity to improve BCSs for thin ewes.

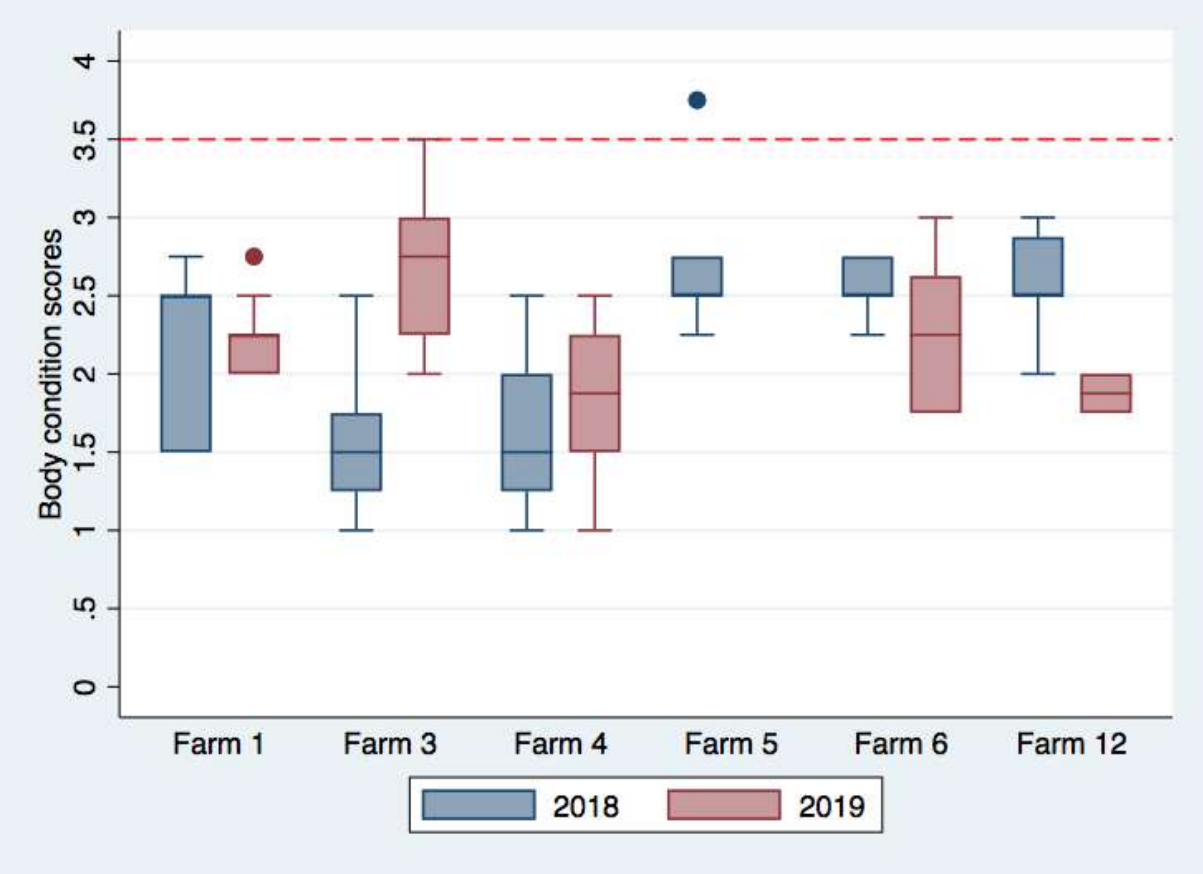


Figure 3: Box and whisker plots of body condition scores for ewes from lowland/improved upland farms presented pre-tupping in Autumn 2019, with 2018 data supplied for comparison. The red dashed line reflects the target BCS for ewes for these farms.

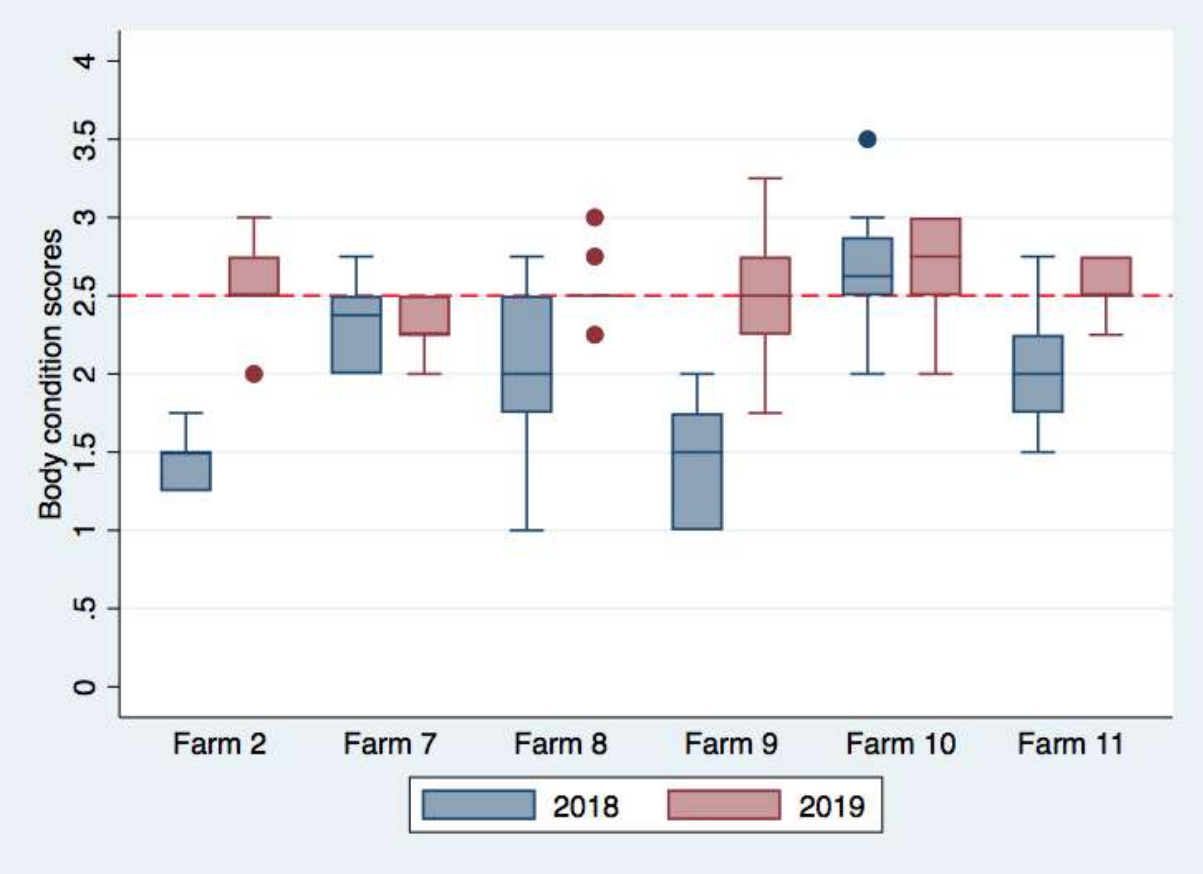


Figure 4: Box and whisker plots of body condition scores for ewes from upland/hill farms presented pre-tupping in Autumn 2019, with 2018 data supplied for comparison. The red dashed line reflects the target BCS for ewes for these farms.

Blood indicators of energy and protein status

For all the farms (Table 17) the mean BOHB concentrations were well within the normal range, however both the urea and NEFA concentrations indicated that the ewes may be under supplied with energy on nine farms, which may be reflective of the sustained metabolic demand up to weaning, compounded by a below optimum BCS up to lambing with limited opportunity for recovery. Only the results from farm 6 suggested the ewes were adequately supplied with energy for their current needs.

Albumin was low on nine farms and *F. hepatica* eggs were detected in pooled faecal samples from six of these farms, as well as one other with normal albumin concentrations. This finding was initially surprising given the timing of the sampling, and that all the farms had treated the ewes with either nitroxynil or closantel after January. There is no known resistance to these particular flukicides yet, although resistance is theoretically possible. However, following discussions with Professor Diana Williams (professor of parasitology, University of Liverpool) about this finding it was considered that it was most likely as a result of re-infection of the ewes with overwintered metacercariae. This is unusual but possible during mild wet winters as

had occurred during the winter of 2018-2019, and anecdotally many more farms in the region experienced similar findings. Ewes on the affected farms were treated with albendazole at a dose of 7.5 mg/kg.

Analysis for Johnes antibodies was not carried out, but this is recognised as a cause of low albumin in clinically affected sheep and the disease is widely under-recognised due in part to its insidious onset (11). As before, all farms were advised to consider Johnes disease as a possibility in cases of chronic weight loss even when well supplied with food and to consider investigating ewes that die at postmortem or culled due to lack of body condition.

The total protein on all the farms was normal or in four cases high. The total protein fraction is primarily a combination of albumin and globulin proteins. The elevated total protein concentrations and normal total proteins where albumin is low could be due to elevated immunoglobulins, possibly due to the *F. hepatica* and *Paramphistome* spp. detected, nematodes or other infectious agents.

Parasitism

As mentioned above, pooled faecal samples were obtained at examination and analysed for the presence of adult *Fasciola hepatica* eggs, *Paramphistome* spp. eggs and gastrointestinal nematode eggs as before. From these samples, seven farms had *Fasciola hepatica* eggs (fluke infection), three farms had *Paramphistome* spp. eggs (rumen fluke infection) and one farm had a moderately high nematode faecal egg count. Using these data, together with detailed historical trends supplied by each farmer, and making use of the parasite forecast available from NADIS, individual parasite control advice was formulated.

Farm ID	BOHB (mmol/l) mean (SD) interp	Urea (mmol/l) mean (SD) interp	NEFA (mmol/l) mean (SD) interp	Albumin (g/l) mean (SD) interp	Total protein (g/l) mean (SD) interp	Presence of *F. hepatica* eggs/*Paramphistome* spp. eggs
1	0.34 (0.08) [0.22-0.44] normal	6.67 (1.54) [4.40-9.20] normal	0.43 (0.57) [0.08-1.83] **above normal**	27.18 (1.25) [25.10-29.60] **low**	84.92 (6.60) [77.00-97.30] normal	**Yes** – No
2	0.39 (0.09) [0.27-0.53] normal	14.4 (2.98) [9.70-19.00] **high**	0.24 (0.13) [0.11-0.52] normal	30.40 (1.91) [28.40-33.60] normal	90.85 (5.59) [82.10-97.30] **high**	No – No
3	0.33 (0.08) [0.21-0.45] normal	9.19 (2.59) [5.30-12.00] **high**	0.25 (0.27) [0.07-0.88] normal	27.40 (2.23) [23.20-30.00] **low**	87.14 (5.35) [80.60-94.30] normal	**Yes** – **Yes**
4	0.42 (0.12) [0.31-0.66] normal	10.58 (0.94) [9.30-12.20] **high**	1.01 (0.30) [0.56-1.51] **above normal**	24.63 (2.76) [21.00-28.50] **low**	88.46 (12.00) [68.70-101.70] **high**	**Yes** – **Yes**
5	-	-	-	-	-	-
6	0.30 (0.09) [0.15-0.43] normal	6.28 (1.59) [4.50-9.20] normal	0.21 (0.25) [0.02-0.76] normal	28.34 (2.29) [24.30-30.90] **low**	83.18 (6.73) [78.10-94.50] normal	**Yes** – No
7	0.38 (0.07) [0.30-0.47] normal	10.41 (1.56) [8.60-12.90] **high**	0.23 (0.12) [0.07-0.42] normal	28.48 (1.97) [26.10-31.00] **low**	91.16 (8.31) [80.30-101.70] **high**	No – **Yes**
8	0.47 (0.10) [0.36-0.63] normal	9.43 (1.54) [7.70-12.70] **high**	0.12 (0.05) [0.06-0.21] normal	27.84 (2.23) [25.00-32.00] **low**	85.70 (7.11) [73.80-96.00] normal	No – No
9	0.55 (0.08) [0.48-0.69] normal	11.00 (1.11) [9.30-12.70] **high**	0.30 (0.23) [0.12-0.81] normal	28.73 (1.19) [27.90-31.30] **low**	87.70 (5.59) [78.80-95.00] normal	No – No
10	0.34 (0.08) [0.21-0.44] normal	10.14 (1.43) [8.70-13.30] **high**	0.16 (0.09) [0.05-0.33] normal	29.55 (1.06) [27.90-30.90] **low**	92.91 (6.40) [86.50-104.20] **high**	**Yes** – No
11	0.33 (0.06) [0.23-0.42] normal	8.58 (1.45) [6.50-10.50] **high**	0.18 (0.09) [0.08-0.32] normal	30.89 (1.78) [28.80-34.30] normal	84.83 (4.22) [80.60-93.10] normal	**Yes** – No
12	0.48 (0.17) 0.30-0.79] normal	9.1 (0.94) [7.80-10.90] **high**	0.94 (0.58) [0.37-2.07] **above normal**	26.09 (1.67) [24.20-29.80] **low**	85.43 (6.77) [77.30-95.70] normal	**Yes** – No
Reference ranges	<0.8 mmol/l	2.8-7.1 mmol/l	<0.40 mmol/l	30-48 g/l	60-79 g/l	

Table 17: Blood indicators of energy and protein status for ewes sampled from 11 of the 12 farms post-weaning and pre-tupping in Summer 2019. Interpretations of the blood analytes are based on ranges supplied by the NUVetNA laboratory.

Blood haematology data

As in 2018, a similar amount of blood haematology data were produced from the blood samples collected. Summary data are supplied (Table 18).

Seven flocks each had at least one sheep sampled with a mild anaemia. Those sheep below the threshold were only just below in all cases except one; one sheep from flock three had a marked anaemia with a haematocrit of 19.7%. Again, no specific reason for this was found at either flock or individual level. However, as in 2018, this anaemia was typically (although not in every case) non-regenerative and as such could well be as a result of a prolonged period of inadequate nutrition. Also, due to the coughing observed in 2018, these farmers were alerted to be vigilant for any clinical disease e.g. an outbreak of coughing/pneumonia in case these individuals were sentinels for an underlying disease process which may be exacerbated by any stress or local environmental changes e.g. inclement weather.

Again, whilst some flocks had small proportions of sheep with either elevated or reduced numbers of circulating white blood cells (which can crudely indicate evidence of a systemic infection), all flocks had sheep with one or other of the specific cell types elevated. Again, some of this e.g. changes in the eosinophils, could be explained by the presence of parasites, detected in the faeces, or in the case of monocytosis the presence of lame sheep, but not in all cases. The farmers were urged to be vigilant for as yet undetected disease and to address the underlying detected problems observed.

Farm ID	N (%) sheep with low HCTs		N (%) sheep with elevated total WBCs		N (%) sheep with below normal total WBCs		N (%) sheep with elevated neutrophils		N (%) sheep with elevated lymphocytes		N (%) sheep with elevated monocytes		N (%) sheep with elevated eosinophils		N (%) sheep with elevated basophils	
1	**1/11**	**(9.1)**	**5/11**	**(45.5)**	0/11	(0.0)	**2/12**	**(16.7)**	**1/12**	**(8.3)**	**12/12**	**(100)**	**6/12**	**(50.0)**	**4/10**	**(40.0)**
2	0/8	(0.0)	**1/8**	**(12.5)**	0/8	(0.0)	**3/9**	**(33.3)**	**1/9**	**(11.1)**	**6/9**	**(66.7)**	**1/9**	**(11.1)**	**2/6**	**(33.3)**
3	**2/7**	**(28.6)**	0/0	(0.0)	0/0	(0.0)	**3/10**	**(30.0)**	**3/10**	**(30.0)**	**9/10**	**(90.0)**	**4/10**	**(40.0)**	**3/6**	**(50.0)**
4	0/7	(0.0)	0/0	(0.0)	0/0	(0.0)	**2/9**	**(22.2)**	**2/9**	**(22.2)**	**6/9**	**(66.7)**	**4/9**	**(44.4)**	**3/9**	**(33.3)**
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	**4/6**	**(66.7)**	**1/6**	**(16.7)**	0/0	(0.0)	**3/8**	**(37.5)**	**2/8**	**(25.0)**	**8/8**	**(100)**	**2/8**	**(25.0)**	**3/6**	**(50.0)**
7	0/7	(0.0)	0/0	(0.0)	0/0	(0.0)	**5/12**	**(41.7)**	**5/12**	**(41.7)**	**10/12**	**(83.3)**	**5/12**	**(41.7)**	**8/12**	**(66.7)**
8	**2/8**	**(25.0)**	0/0	(0.0)	**1/7**	**(14.3)**	**2/9**	**(22.2)**	**2/9**	**(22.2)**	**8/9**	**(88.9)**	**2/9**	**(22.2)**	**2/7**	**(28.6)**
9	**2/7**	**(28.6)**	0/0	(0.0)	**1/7**	**(14.3)**	**5/12**	**(41.7)**	**5/12**	**(41.7)**	**6/12**	**(50.0)**	**5/12**	**(41.7)**	**8/11**	**(72.7)**
10	0/8	(0.0)	0/0	(0.0)	**1/7**	**(14.3)**	**1/8**	**(12.5)**	**1/8**	**(12.5)**	**5/8**	**(62.5)**	**2/8**	**(25.0)**	**1/6**	**(16.7)**
11	**1/8**	**(12.5)**	0/0	(0.0)	0/0	(0.0)	**2/10**	**(20.0)**	**2/10**	**(20.0)**	**9/10**	**(90.0)**	**2/10**	**(20.0)**	**2/6**	**(33.3)**
12	**3/7**	**(42.9)**	**2/7**	**(28.6)**	0/0	(0.0)	**3/9**	**(33.3)**	**2/9**	**(22.2)**	**9/9**	**(100)**	**2/9**	**(22.2)**	**2/7**	**(28.6)**
Reference ranges	27.0-42.0%		5.06-14.12x10^9/L				1.17-6.11x10^9/L		2.54-9.60x10^9/L		0.1-1.01x10^9/L		0.05-0.95x10^9/L		0.0-0.12x10^9/L	

Table 18: Haematology data from the sheep sampled post-weaning/pre-tupping in Summer 2019. Interpretations are based on ranges supplied by IDEXX laboratories where appropriate and are suitable for the analyser used and for sheep.

Trace elements

Further liver biopsies and blood samples were taken from a sample of ewes post-weaning/pre-tupping, along a similar line of investigation to that of 2018. The purpose of this was twofold: 1) in order to evaluate the trace element status of the ewes to see if a similar intervention as in 2018 was required; 2) to monitor the response of the ewes to the trace element interventions already given. In 2019, sampling was carried out a little earlier than in 2018 allowing more time to make adjustments and plan supplementation as necessary.

From an analysis of the data, the flocks were diagnosed as either likely or unlikely to benefit from supplementation (Table 19 and Table 20). No changes were made to five farms and one group from farm 7; they were advised to supplement in the same way as in 2018. Four flocks required only minor changes i.e. the removal of one element from the supplementation. Flock four remained the most challenging and is presented here as a case study:

Flock four: case study

Pre-tupping 2018

- Blood and liver tissue results indicated that copper, selenium and zinc were required.
- Forage analysis revealed relatively high levels of sulphur, iron and molybdenum.
- Two sheep showed blood evidence of risk of a thiomolybdate toxicity.
- Long-term (6 months), slow and continuous release copper supplementation would have been ideal i.e. bolus administration, alongside the other necessary elements. However, a multi component trace element drench containing copper, selenium and cobalt had already been given shortly after sampling and prior to the results being known. To give another source of copper at this time could have resulted in copper toxicity.
- As a compromise, a further multi component trace element drench was given at scanning to counteract an expected decline in these elements within the sheep.

Pre-lambing 2019

- The two-drench approach was shown to be inadequate, particularly with regards to copper as evidenced in the pre-lambing blood analyses.
- Due to the alarmingly low results, a multi trace element bolus was given to ewes at lambing as they were to be moved to pasture with high concentrations of molybdenum and sulphur for a prolonged period immediately after lambing.
- It was expected that the copper would be utilised and excreted within about 6 months and then post-weaning/pre-tupping in the autumn a similar bolus could be given to provide better supplementation for the pregnant ewes.

- In addition, tests (out with this project) carried out on barren ewes indicated that 3/8 had recent exposure to *Toxoplasma gondii*, a protozoan parasite associated with foetal resorption and abortion. It was considered very likely that some of the below optimal scanning results and increased proportion of barren ewes was due to this (21). Vaccination was recommended for breeding ewes prior to the next breeding cycle.

Pre-tupping 2019

- Whilst some ewes were predictably deficient in copper, some demonstrated very high copper concentrations within the liver tissue. This was concerning and further supplementation could have resulted in copper toxicity.
- Appropriate supplementation could not be carried out this time, due to safety concerns, however re-investigation post-weaning/pre-tupping 2020 will be carried out, and, if as expected blood and liver tissue concentrations indicate a deficiency again, then supplementation could be carried out pre-tupping, with likely improved health and production benefits.

Farm ID	Liver tissue and blood results												Advice
	TISSUE Copper µmol/kg DM mean (SD) [range] *interpretation*	BLOOD Plasma copper µmol/l	BLOOD Serum Caeruloplasmin mg/dl mean (SD) [range] *interpretation*	BLOOD Superoxide dismutase U/g Hb mean (SD) [range] *interpretation*	TISSUE Selenium µmol/kg DM mean (SD) [range] *interpretation*	BLOOD Plasma selenium µmol/l mean (SD) [range] *interpretation*	BLOOD Glutathione peroxidase U/ml PCV mean (SD) [range] *interpretation*	Status change: Glutathione peroxidase/ Plasma selenium	TISSUE Cobalt µmol/kg DM mean (SD) [range] *interpretation*	BLOOD Plasma cobalt µmol/l mean (SD) [range] *interpretation*	BLOOD Plasma zinc µmol/l mean (SD) [range] *interpretation*	TISSUE Manganese µmol/kg DM mean (SD) [range] *interpretation*	Black: no change from 2018; Red: change from previous practice
1 a	3352 (1475) [1910-6399 *normal*	14.6 (3.6) [11.5-23.0] *normal*	32.6 (8.0) [21.6-48.5] *normal*	1973 (149) [1763-2194] *marginally low*	5.2 (1.2) [3.9-7.6] *marginally deficient*	0.53 (0.08) [0.45-0.66] *sub-optimal*	209 (85) [90-345] *marginally high*	Down	2.70 (1.79) [1.61-7.06] *normal*	4.6 (0.4) [4.1-5.2] *marginally low*	11.9 (1.2) [10.5-14.1] *marginally low*	152.4 (27.0) [116.0-202.9] *normal*	Co Se Zn bolus pre-tupping
1 b	2024 (1654) [169-3641] *normal (1 low)*	12.4 (1.7) [10.2-13.9] *normal*	21.0 (2.0) [19.0-23.5] *normal*	1767 (287) [1371-2049] *low*	6.5 (1.0) [5.0-7.2] *marginally deficient*	0.61 (0.07) [0.51-0.67] *sub-optimal*	121 (43) [75-178] *normal*	Down	3.18 (0.13) [3.04-3.32] *normal*	5.0 [0.7) [4.1-5.9] *marginally low*	11.3 (1.0) [10.2-12.7] *marginally low*	211.2 (42.1) [185.3-274.0] *normal*	Co Se Zn bolus pre-tupping
2	2082 (1462) [291-4495] *normal*	16.5 (3.4) [12.2-22.7] *normal*	31.2 (4.5) [22.7-35.7] *normal*	1957 (417) [1181-2375] *marginally low*	3.3 (0.6) [2.4-4.2] *deficient*	0.39 (0.09) [0.30-0.51] *marginally low*	45 (14) [26-72] *sub-optimal*	No change	3.64 (3.50) [1.43-12.17] *normal*	5.3 (1.2) [4.0-7.7] *normal*	12.7 (3.4) [8.1-20.2] *normal*	234.1 (50.0) [154.6-316.8] *normal*	Co Se Zn bolus pre-tupping
3	4532 (2876) [1964-10028] *normal*	13.9 (2.4) [10.0-17.8] *normal*	26.9 (8.6) [17.6-45.9] *normal*	2175 (318) [1580-2730] *normal*	6.2 (2.3) [3.6-9.7] *marginally deficient*	0.62 (0.13) [0.46-0.81] *sub-optimal*	153 (68) [71-245] *normal*	Down	2.43 (1.15) [1.06-4.09] *below normal/normal*	4.3 (0.4) [3.7-5.1] *marginally low*	11.0 (1.7) [9.0-14.0] *marginally low*	272.1 (144.3) [93.2-514.8] *normal*	Co Se Zn bolus pre-tupping
4	5033 (4577) [220-13897] *normal (extreme range)*	12.7 (5.7) [4.7-24.9] *normal (wide range)*	29.0 (9.8) [14.9-49.5] *normal*	2174 (329) [1648-2639] *normal*	5.9 (1.8) [3.8-8.9] *marginally deficient*	0.58 (0.14) [0.35-0.78] *sub-optimal*	166 (60) [100-278] *normal*	Down	3.48 (0.53) [2.36-3.96] *normal*	9.1 (1.1) [7.8-10.4] *normal*	12.7 (1.6) [9.4-14.3] *normal*	180.8 (40.3) [123.1-234.4] *normal*	Cu Co Se drench pre-tupping and scanning[1]
6	1165 (804) [224-2093] *below normal*	13.5 (2.6) [9.4-17.3] *normal*	23.5 (2.8) [18.4-27.3] *normal*	1809 (286) [1554-2473] *marginally low*	2.9 (0.5) [2.4-3.8] *deficient*	0.33 (0.06) [0.23-0.40] *marginally low*	48 (18) [28-76] *sub-optimal*	Down	3.19 (0.83) [1.66-4.19] *normal*	5.6 (0.7) [4.6-6.3] *normal*	12.7 (1.9) [10.5-16.3] *normal*	181.3 (33.8) [126.9-223.4] *normal*	Se drench twice; pre-tupping and at scanning.

Table 19: Summary trace element findings and advice given pre-tupping 2019, farms 1-6 (farm 5 had dropped out of the project by this stage). Interpretations of the analytes are based on ranges supplied by the NUVetNA laboratory. For Farm 1, 'a' denotes group 1 (yearling ewes) and 'b' group 2 (adult ewes). Graphical representations of these data are displayed in the appendix.

[1] This approach is likely to be inadequate for some sheep but was taken due to the surprisingly high copper concentrations in some of the liver tissue samples as a result of the bolus administration in the spring (see above). In a years' time it would be expected that much of the excess copper in those sheep with high liver tissue copper concentrations would have been excreted. A shorter acting bolus could then be used to reduce workload (compared to two drenches) and also to provide a more consistent supply of trace elements.

Farm ID	Liver tissue and blood results												Advice
	TISSUE Copper µmol/kg DM mean (SD) [range] *interpretation*	BLOOD Plasma copper µmol/l	BLOOD Serum Caeruloplasmin mg/dl mean (SD) [range] *interpretation*	BLOOD Superoxide dismutase U/g Hb mean (SD) [range] *interpretation*	TISSUE Selenium µmol/kg DM mean (SD) [range] *interpretation*	BLOOD Plasma selenium µmol/l mean (SD) [range] *interpretation*	BLOOD Glutathione peroxidase U/ml PCV mean (SD) [range] *interpretation*	Status change: Glutathione peroxidase/ Plasma selenium	TISSUE Cobalt µmol/kg DM mean (SD) [range] *interpretation*	BLOOD Plasma cobalt µmol/l mean (SD) [range] *interpretation*	BLOOD Plasma zinc µmol/l mean (SD) [range] *interpretation*	TISSUE Manganese µmol/kg DM mean (SD) [range] *interpretation*	Black: no change from 2018; Red: change from previous practice
7 a	1921 (1078) [581-3219] *normal (1 low)*	15.7 (3.8) [11.8-20.7] *normal*	25.7 (4.6) [19.3-29.5] *normal*	1884 (243) [1572-2164] *marginally low*	15.7 (4.3) [10.7-20.2] *normal*	2.06 (0.23) [1.77-2.34] *high*	466 (27) [430-494] *high*	Down	2.59 (1.14) [1.22-3.99] *normal*	5.9 (1.5) [4.9-8.1] *normal*	12.1 (1.1) [11.0-13.2] *normal*	164.7 (63.1) [102.8-241.1] *normal*	Se drench at scanning[2]
7 b	3262 (1293) [1539-4652] *normal*	13.5 (3.8) [9.7-18.7] *normal*	21.9 (4.0) [18.1-27.5] *normal*	2027 (233) [1732-2230] *normal*	6.3 (2.1) [4.2-8.6] *marginally deficient*	0.71 (0.20) [0.49-0.97] *sub-optimal*	193 (70) [125-283] *marginally high*	Down	2.19 (0.34) [1.88-2.68] *normal*	5.1 (0.2) [4.9-5.3] *normal*	11.9 (1.4) [10.2-13.3] *marginally low*	173.4 (14.0) [156.1-186.7] *normal*	Co Se Zn bolus pre-tupping
8	2881 (1571) [372-4965] *normal (1 low)*	12.9 (0.7) [12.0-13.9] *normal*	21.9 (2.9) [18.2-27.6] *normal*	2007 (141) [1840-2228] *marginally low/normal*	4.2 (0.9) [2.7-5.3] *deficient*	0.54 (0.11) [0.34-0.66] *sub-optimal*	123 (64) [35-196] *normal (wide range)*	Down	3.08 (1.33) [1.18-4.92] *normal*	7.1 (2.3) [4.4-10.9] *normal*	11.6 (2.1) [8.9-14.7] *marginally low*	182.8 (49.3) [103.1-259.9] *normal*	Co Se Zn bolus pre-tupping; Cu not required
9	2884 (2671) [228-7368] *normal (wide range)*	13.4 (2.6) [8.0-17.3] *normal*	24.5 (5.4) [14.2-31.7] *normal*	1803 (469) [1091-2301] *marginally low (wide range)*	6.3 (2.2) [2.4-9.7] *marginally deficient*	0.71 (0.18) [0.52-0.93] *sub-optimal*	145 (60) [83-243] *normal*	Down	2.34 (0.68) [1.49-3.43] *normal (below normal)*	5.1 (2.3) [3.2-10.6] *normal*	10.1 (1.3) [8.2-12.1] *marginally low*	155.5 (40.4) [64.6-191.6] *normal*	Se Co Zn could be useful mid-pregnancy
10	3013 (1721) [1217-6424] *normal*	14.9 (1.7) [12.2-17.0] *normal*	31.4 (6.5) [23.6-41.5] *normal*	1860 (221) [1596-2276] *marginally low*	14.5 (2.4) [11.9-19.2] *normal*	1.93 (0.20) [1.68-2.31] *marginally high*	444 (86) [316-558] *high*	Down	2.51 (0.30) [2.08-3.10] *normal*	7.0 (1.4) [5.3-9.4] *normal*	13.6 (1.1) [11.7-14.9] *normal*	291.1 (48.4) [201.0-349.0] *above normal*	No supplementation required
11	1809 (1205) [460-3575] *normal*	15.2 (1.7) [12.6-17.6] *normal*	25.6 (3.0) [21.1-28.7] *normal*	1598 (285) [1043-1976] *low*	5.8 (0.9) [4.4-6.9] *marginally deficient*	0.77 (0.25) [0.51-1.30 *sub-optimal*	251 (92) [61-340] *high*	Down	3.40 (0.83) [2.66-5.05] *normal*	9.8 (5.2) [4.9-16.7] *normal*	12.3 (1.2) [9.8-13.3] *normal*	185.3 (32.0) [147.2-234.7] *normal*	Cu[3] Co Se bolus pre-tupping; Zn[4] not required
12	3568 (2150) [1376-8011] *normal*	14.6 (1.7) [12.4-17.4] *normal*	31.3 (3.9) [26.6-37.8] *normal*	2176 (218) [1952-2533] *normal*	5.4 (1.0) [4.0-6.7] *marginally deficient*	0.42 (0.06) [0.32-0.52] *sub-optimal*	107 (43) [60-185] *normal*	Down	2.36 (0.60) [1.82-3.68] *normal*	5.8 (1.6) [4.2-8.6] *normal*	14.2 (1.7) [12.4-17.1] *normal*	350.6 (74.3) [237.5-478.9] *high*	Se Co bolus pre-tupping; ZN[4] not required

Table 20: Summary trace element findings and advice given pre-tupping 2019, farms 7-12. Interpretations of the analytes are based on ranges supplied by the NUVetNA laboratory. For Farm 7, 'a' denotes group 1 (BFL x Tex) and 'b' group 2 (Welsh). Graphical representations of these data are displayed in the appendix.

[2] Selenium not required pre-tupping, although a decline was expected over the winter; a drench around scanning should provide sufficient selenium to cover the last part of pregnancy.

[3] These results indicate only a mild/moderate deficiency in copper; supplementation may or may not be beneficial. Given the success of supplementation in previous years with a six-month bolus, a suitable wash out period and potential under nutrition during pregnancy if weather conditions are severe, then on balance, copper supplementation may have some benefit.

[4] Zinc not required but if present in bolus is unlikely to have any adverse effects.

Over the next series of pages, the sheep data for each farm are presented as a series of graphs (one for each trace element analyte) indicating the mean for each analyte together with the standard deviation (SD). This may help give a visual guide as to how each analyte fluctuated over time, together with an estimate of the spread of the data for each group of samples. The horizontal red lines are added to each graph to indicate the optimal or normal reference range as specified by NuVETNA laboratories.

There may be some difficulties in comparisons between farms in that on occasion, in order to display the data clearly, the y axis scale has had to be adjusted. This is due to some of the large differences observed between the concentrations of some analytes between farms.

Objective 3 - Knowledge exchange

The data and information gathered for each farm was throughout supplied back to each farmer through verbal discussion and written reports.

Three broad questions were considered in understanding the applicability of these techniques in the Welsh sheep farming context:

1) *Would a deficiency/oversupply of copper, selenium and cobalt have been identified solely from blood analysis without additional liver tissue sampling?*

Copper

The data in Table 5 and Table 6 detail the trace element results taken pre-tupping in 2018 at the start of the project. In this analysis, for the majority of flocks the interpretation from the blood results would have been sufficient, however the inclusion of the liver tissue samples was useful in flocks 1 and 4 in adding detail to aid in their interpretation.

In flock one, plasma copper was marginally low, although caeruloplasmin and SOD were within normal ranges. The inclusion of the liver tissue concentration helped confirm that the marginally low plasma copper was less significant in the longer term if grazing were to continue as expected. As it turned out, with regard to this flock, the monitoring blood results taken pre-lambing (Table 15) demonstrated alarmingly low plasma copper, caeruloplasmin and SOD concentrations, with samples from neonatal lamb livers showing varying results (Table 16). In the future, some copper supplementation could well be beneficial for optimum production.

In flock 4, plasma copper again was only marginally low, although SOD was low. The inclusion of the liver tissue samples helped identify a potential deficiency which when combined with the forage analysis provided information that meant supplementation would likely be beneficial. The preferred method of administration of the copper would have been a slow release preparation e.g. bolus, however the farmer gave a drench prior to the results becoming available. Further supplementation at this time with another copper containing product could have resulted in toxicity. This approach however was shown to be inadequate by the very low blood copper concentrations detected later, at the pre-lambing analysis (Table 15 and Table 16). In essence, this served to confirm suspicions that slow release supplementation would have been more beneficial.

In conclusion, where marked deficiencies are present, and continuous, blood analysis is likely to be sufficient. However, where sheep graze pastures with varying amounts of copper and its antagonists, multiple measures appear to give a better depth of

information thereby facilitating interpretation and guiding intervention decisions over the longer term.

Selenium

For selenium, when considering the same data (Table 5; Table 6) the answer is much clearer; for many of the flocks the blood analysis would have been inadequate. For flocks 1, 2, 3, 6, 7, 8 and 12, blood analyses indicated only a sub-optimal selenium status, however the liver tissue confirmed a deficiency or marginal deficiency. Taken with the forage analysis, these data were extremely useful in confirming the need for selenium supplementation prior to tupping; blood analysis alone could have resulted in under supplementation on many farms.

Cobalt

In general, the liver tissue and blood analyses would have been interpreted similarly. There are a number of exceptions however, for example flock 7 and 8 both showed liver tissue results that indicated a lower cobalt status compared to the blood analysis (Table 6 and Table 11). Also, flocks 3, 6 and 9 showed marginally low blood concentrations but normal liver concentrations. Taking the two together was useful in identifying situations where cobalt may have been undersupplied either historically or currently.

2) Would the nutritional advice have been different had tissue sampling not been carried out?

The answer to this question is complex and varies from flock to flock. For several flocks there would likely have been no difference, although this was impossible to predict at the outset of the project. However, for some flocks, the additional data was useful to explore the potential benefits of supplementation, or not, against the risks. For example, the data for pre-tupping samples in 2018 for flock 3 (Table 5) showed marginally low plasma copper concentrations (mean 9.8 µmol/l (SD 2.2)) but an above normal liver tissue concentration (mean 6075 µmol/kg DM (SD 3052)). Similarly the selenium concentration was within the normal range in the blood (mean 0.59 µmol/l (SD 0.10)), as was the concentration of glutathione peroxidase (156.3 U/ml PCV (SD 56.0)), however the concentration within the liver was marginally deficient (6.97 µmol/kg DM (SD 0.94)). The additional information therefore for this flock was useful in determining the need and safety when considering supplementation. Indeed, both copper and selenium supplementation had been given in previous years, however further copper supplementation could have resulted in toxicity if this had continued, although selenium supplementation was considered to be still beneficial. Using just the blood results for copper whilst not indicative of a deficiency *per se* could have indicated some potential benefit from a small amount of copper, and with regard to selenium the results could have indicated a sufficient assimilation and no supplementation, which would likely have become sub-optimal.

In another example, samples from flock 2 taken pre-tupping in 2019 (Table 19) showed only marginally low plasma selenium concentrations (mean 0.39 µmol/l (SD 0.09)) and sub-optimal glutathione peroxidase concentrations 45 U/ml PCV (SD 14)) but deficient liver concentrations (mean 3.3 µmol/kg DM (SD 0.6)). Consequently, the marginally low blood results could have led to a cost-benefit decision not to supplement when actually over the pregnancy period the ewes were likely to require more selenium than was available and have insufficient tissue reserves to be able to support this. This could also have consequent effects in their lambs as well.

Conclusions

Considering the experiences detailed throughout this report, I would on balance suggest that liver tissue samples are likely to be useful particularly during an initial investigation and monitoring phase, as was the situation for these flocks in this project. This would be particularly useful where sheep farmers do not know the underlying ability of *their* sheep to assimilate trace elements, or their response to supplementation. Moving forward they could be useful on a case by case basis to monitor and assess the response to supplementation/no supplementation. For example, for flock 4, sampling is likely to continue until the situation with regard to copper stabilises and where a practical system for the right amount of supplementation can be demonstrated to be achieved.

Where results indicate that a flock nutritional situation is relatively stable, then further tissue sampling may not be of benefit. For example, the data for flock 10, revealed that trace elements were assimilated to roughly within optimal concentrations within blood and tissue and this remained similar between the two sampling periods. Further testing was unlikely to bring any benefits unless changes were made to the system or breed of sheep used.

As ever, where severe deficiencies occur, blood sampling alone will continue to be useful, however where optimal production is the goal, rather than the investigation of a clinical disease or syndrome, then tissue sampling is likely to be of benefit for many flocks due to the added depth of information this brings. It is likely to bring particular benefits where sheep change pastures regularly and where the pastures are of differing mineral concentrations. In addition, it is also likely to be useful, as found in this project, in situations where borderline cases are identified in that the addition of the extra data enables a more informed and therefore more nuanced decision.

3) Would the OG participants consider the additional commercial cost of this detailed analysis of a panel of nutritional markers beneficial over a more traditional blood sampling approach of a limited number of nutritional markers?

The commercial costs to the farmer for these investigations as carried out in this project are as follows; all costs are detailed exclusive of VAT and are calculated at 2020 prices. The laboratory costs quoted are those for NuVETNA laboratories and the veterinary time and parasitology tests quoted are those for Wern Veterinary Surgeons. The haematology costs have been excluded as they would not normally be a part of a nutritional analysis; they were included in the project in order that a thorough and detailed investigation could be carried out.

Post weaning/pre tupping investigation

Cost	Price	Total
Examination, blood and tissue sampling for 8 ewes		
Veterinary fee (examination and sampling fees)	£113.21	£113.21
Laboratory fees (energy, protein and trace elements)	£30 submission fee[5] £32 per blood sample[6] £21.50 per liver tissue sample	£458.00
Faecal parasite analysis on pooled samples[7]	Liver and rumen fluke egg identification £26.25 Gastrointestinal nematode count £12.58	£38.83
Advice and report	£113.21	£113.21
Total		**£723.25**

Table 21: Costs associated with the tests and investigations carried out post-weaning/pre-tupping.

[5] Costs can be saved here if farmers group together in that a submission fee could cover more than one farm if samples are sent together.
[6] Costs can be saved here if energy and protein analyses are not required or if just energy or just protein is required.
[7] These costs may also be saved if farmers already have comprehensive parasite control plans already active.

Pre-lambing monitoring

Cost	Price	Total
Examination and blood sampling for 15 ewes		
Veterinary fee (examination and sampling fees)	£113.21	£113.21
Laboratory fees (energy, protein and trace elements)	£30 submission fee[8] £32 per blood sample[9]	£350.00
Advice and report	£113.20	£113.21
Total		**£576.42**

Table 22: Costs associated with the tests and investigations carried out pre-lambing.

There would also be a need to consider the costs associated with forage sampling, for which the number of samples could vary widely between farms, depending on the structure of the farm. Currently sample analysis costs are in the region of £26.00 per sample.

Therefore, for two post-weaning/pre-tupping investigations and one pre-lambing investigation, together with three forage samples the total commercial cost could be £2100.92. However, there are several ways these costs could be reduced, although there would likely be a loss of potentially useful data in some instances. For farmers considering such investigations they need to weigh this up against the costs they already incur against any potential benefits.

For example, an unnecessary bolus could cost in the region of £1 per ewe which if necessary, is likely to deliver a return, yet if not, is an unnecessary cost. Similarly, over-supplying ewes with concentrate feed pre-lambing can result in a large feed bill, together with consequences such as an increased number of large lambs and fat ewes with consequent dystocia and its results. Conversely, under supplying concentrate feed pre-lambing is likely to result in reduced milk quality and quantity – with consequent disease consequences for both ewes and lambs, increase the number of ewes with difficulties lambing, and, potentially lead to pregnancy toxaemia and its consequences in some ewes.

[8] Costs can be saved here if farmers group together in that a submission fee could cover more than one farm if samples are sent together.
[9] Costs can be saved here if energy and protein analyses are not required or if just energy or just protein is required.

This cost benefit analysis requires considerable thought on the part of each farmer and is likely to have different conclusions associated with differences in individual circumstances. Additionally, these figures have been calculated based on sampling a flock managed as one unit. If flocks are managed in separate groups with differing land structures and management then this also needs to be taken into account and sampling figures adjusted accordingly.

Discussion of the results and lessons learned from the use of this investigative and management approach

Body condition

Throughout this project body condition scoring was used as a crude general marker of overall ewe health. It is well correlated to many production markers and there is much advice available around maintaining optimum body condition for production e.g. AHDB Beef and Lamb (1), AHDB Beef and Lamb (10). Most of the farmers in this study were aware of body condition scoring although most were not carrying it out in a routine or standardised way. Training was provided throughout this study to all farmers in scoring ewes and in setting targets for different stages of production, together with approaches to achieve those targets e.g. forming grazing groups of ewes in similar body condition as well as investigating, understanding and addressing underlying health needs. Some of the farmers are now regularly body condition scoring ewes at key points in the production cycle e.g. weaning, pre-tupping, scanning, pre-lambing etc. This has helped with planning the grazing and adjusting the feed appropriately.

In 2018, body condition scores were generally well below targets, which was considered to be mainly due to the lack of grass available as a result of an unusually dry summer (Figure 2). By summer/autumn 2019, these had generally improved relative to 2018 (Figure 4) and although some flocks were still below key production targets, the time and forage available was generally sufficient to allow adjustment prior to tupping.

Scanning results

Ultrasound scanning can be used to detect the number of unborn foetuses approximately halfway through pregnancy. Many factors can impact on conception and the survival of the foetus in early pregnancy, including the body condition of the ewe prior to and during pregnancy; the general availability of food during breeding, together with the metabolic status of the ewe; infectious disease; stress; weather etc. For the flocks in this project, the poor body condition of many of the ewes prior to tupping was likely to have had the greatest effect overall with other factors also playing a role in several cases.

For some flocks, infectious disease was shown to be important and likely to be affecting scanning results with barren sheep from farms 2 and 4 both showing high antibody titres to *T. gondii* in some ewes. This parasite is well known to cause early embryonic death and reabsorption during active infection (22, 23) as well as abortion. In 2019/2020 barren ewes from flock 2 were also tested for antibodies to border disease virus with some ewes showing antibody titres suggestive of previous exposure. This disease can also cause early embryonic death and reabsorption, together with later abortions and is likely to be contributing to the lower number of

lambs scanned as well as effects on neonatal lambs (24, 25). One other flock (farm 7) was diagnosed with *Campylobacter jejuni*. related abortion. Depending upon how long this had been circulating amongst the ewes this may also have affected the scanning result on this farm with early losses going undetected (26).

All flocks were at risk of *F. hepatica* infection and should have been well managed given the advice and planning in place. However, interestingly, 7/11 flocks were diagnosed with adult fluke infections during the summer of 2019/2020 post-weaning. This was surprising but as mentioned previously was considered to be most likely as a result of re-infection of the ewes with overwintered metacercariae. This is unusual but possible during mild wet winters as had occurred during the winter of 2018-2019. Sub-clinical parasitism with *F. hepatica* may have therefore impacted on the poor scanning results for some farms. Moving forward, farmers would be well advised to test their sheep around lambing time to ensure winter treatments for fluke have been efficacious. In doing so they will have the opportunity to reduce the amount of eggs shed on the pasture if further treatments are required.

Trace element management in 2018/2019 was also less than optimum in some cases as shown by the monitoring investigations carried out pre-lambing. Copper was variably managed across the flocks, with concern particularly for flocks 1 and 4. Selenium and cobalt were better managed on average for most flocks, although zinc was less well managed with blood concentrations remaining marginally low or low for all flocks (Table 15). Improving the management of copper on those farms where there was evidence of deficient blood and liver tissue concentrations could serve to improve fertility overall, including conception rates (20). As yet it is unclear if improving the management of zinc is likely to improve conception or the maintenance of pregnancies, however optimal management of this element is likely to have other beneficial effects including improved immune responses of ewes, improved lamb birth weights, less protracted labour and a lower incidence of retained foetal membranes (27), which can all impact on ewe and lamb survival.

Blood indicators of energy and protein status

For optimal nutritional planning utilising body condition scores of ewes is essential and is effective as a planning tool, however the use of blood indicators of energy and protein status can help detect changes not evident from a physical examination and can be used to help refine a feeding plan. In many instances in this project (although not all) energy status was as expected based on the body condition of the ewes at each sampling period, however there were several occasions where protein markers were considerably lower than expected. This was very useful in helping formulate the advice given to each farmer in how to plan the next phase of feeding the ewes. Low albumin was a particular feature for many flocks during the project, indicating a longer-term protein deficit and close to lambing was likely to impact on the milk volume and quality produced, with subsequent impacts on lamb survival. Moving forward, with

better nutritional planning flocks should be better supported and these deficits addressed with consequent improvements in health and production. In addition, in many flocks, parasitism, particularly with liver fluke, could also have played a role and the improved monitoring and planning with regard to this parasite will likely lead to improvements in performance.

Measuring the energy and protein status of ewes toward the end of pregnancy together with concurrent body condition scoring was considered invaluable in the majority of flocks in fine tuning the diet offered. Frequently advice was given to adjust the quantity of concentrate feed given based on the results of the blood markers and in no case was pregnancy toxaemia observed in more than individual isolated incidents, often associated with another adverse event e.g. lameness. In addition, no flock experienced unusual numbers of ewes with mastitis, which can occur when ewes are unable to produce a sufficient volume of milk.

Parasitism

Parasite control is fundamental to any sheep enterprise and is a major concern across the industry. Parasites clearly affect production and can have major adverse welfare effects as well. Some of the data in this project were surprising, for example the frequent identification of adult liver fluke during the postweaning/pre-tupping investigations in 2019. Examples like this highlight the importance of regularly monitoring the health of flocks and the success of any control or management strategies implemented.

Given this finding and the inherent dangers associated with the biopsy of liver tissue, I would strongly recommend either testing the sheep to be sampled for the presence of liver fluke infection (preferably using a copro-antigen test or blood antibody ELSIA test) or prophylactically treating the sample population only, at least three weeks prior to sampling. This way, any risk to the sampled sheep can be reduced by addressing any potential underlying damage to the liver by liver flukes.

Blood haematology data

These data were surprising in the frequency of elevated individual leucocytes. There is relatively little published literature focused on broad investigations of haematological data for sheep which makes the frequency of this finding challenging to interpret. When considering the responses seen in other animal species, we could expect that these elevations would correlate well with obvious observable infections or inflammation however except in isolated individual cases this was not the case in this project. As such, in two instances coughing was later observed in the flocks from which sheep were sampled, and this could have been associated with the leucoyctic changes seen. In addition, some of the effects could be related to parasitism, however it was the frequency and extent of the elevations that was surprising. In the future haematological changes like this should be borne in mind as potentially ‘background’

but also could be suggestive of an undetected infectious challenge or inflammatory change. The coughing outbreaks were interesting as the timing coincided with an increased seasonal risk for pasteurellosis and indeed these flocks have now adjusted the timing of booster vaccinations to account for this potential seasonal risk.

Trace elements

Further discussion of this area of the project is included in the previous section addressing the specific objective (number 3) entitled 'Knowledge exchange'.

In brief, the investigations revealed varying levels of these elements for each farm, indeed each flock was unique in its needs and a flock level approach was essential. The management of the perceived deficiencies was variable and shows the importance of monitoring post intervention. The results from some flocks showed that some trace elements remained below optimal concentrations within blood and/or tissue or in some cases had reached concentrations that were too high and could lead to toxicity. The monitoring tests facilitated appropriate adjustment and understanding of how the sheep for each flock responded to the intervention.

For most flocks, there were several factors identified that needed addressing, for example copper requirements, infectious disease management and parasite control. It is unlikely that management will become optimal within a short period of time due to the complex nature of managing and addressing multiple issues at once. However, identifying and understanding key issues will facilitate change and enable improved management in the future. As each farmer builds on their experiences from this project, they should be able to bring improvements into all the areas identified, including general nutritional management, parasite control, infectious disease control and trace element optimisation.

Overall conclusions and key lessons learned

1. There are big improvements to be made in managing nutrition on sheep farms and a key intervention would be **for farmers to adopt regular body condition scoring**, adjusting grazing to enable sheep to meet pre-established targets at key times of the year.
2. Parasitism remains an important feature of flock management and regular monitoring of control programmes in necessary to ensure they remain effective.
3. Infectious diseases are common causes of production problems; proactive investigation can ensure appropriate action is taken in the future.
4. Trace elements
    a. Trace elements are an important component in proactive and optimal nutritional planning but are far less important when compared to the overall availability of forage and the body condition of the ewes.

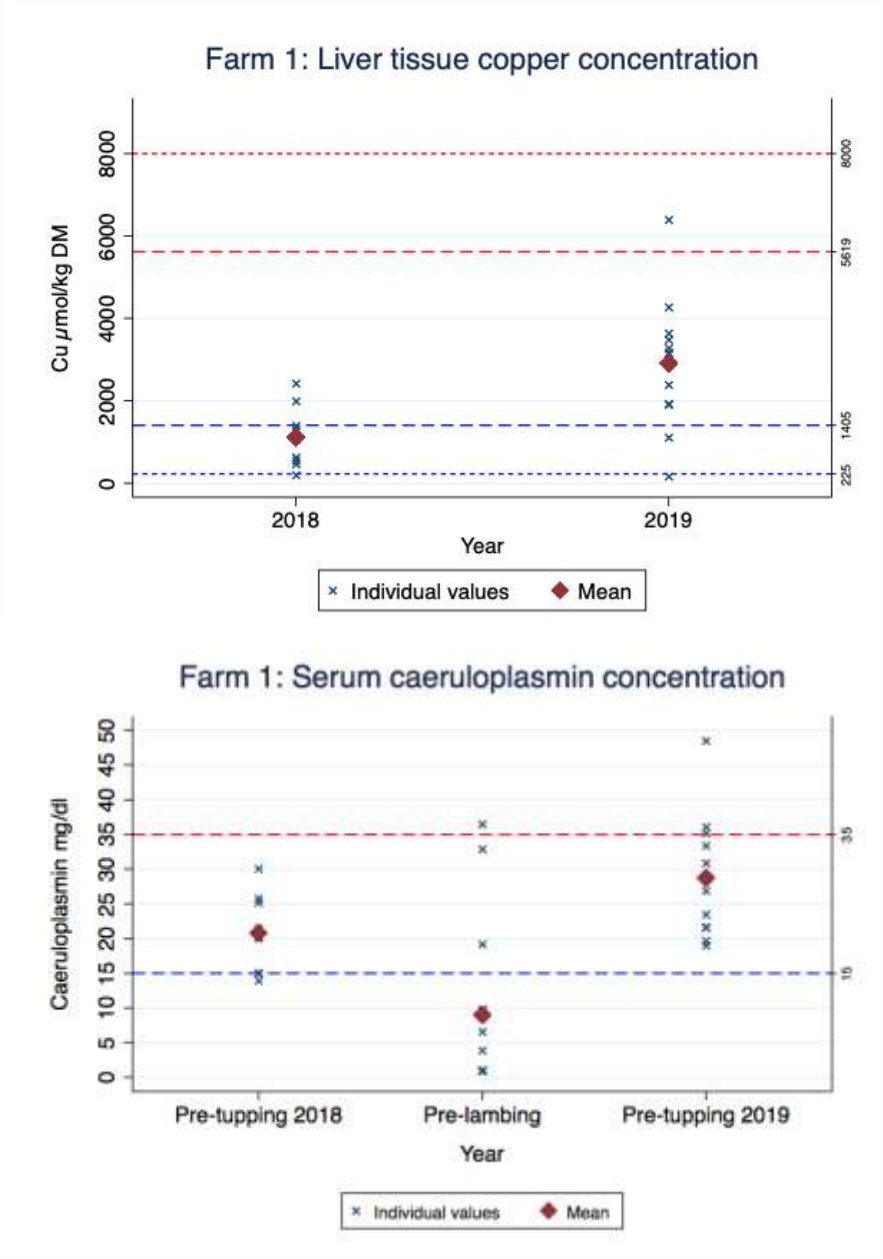
b. The techniques used in this project were extremely useful in determining the trace element needs of the ewes and in monitoring the response to supplementation.

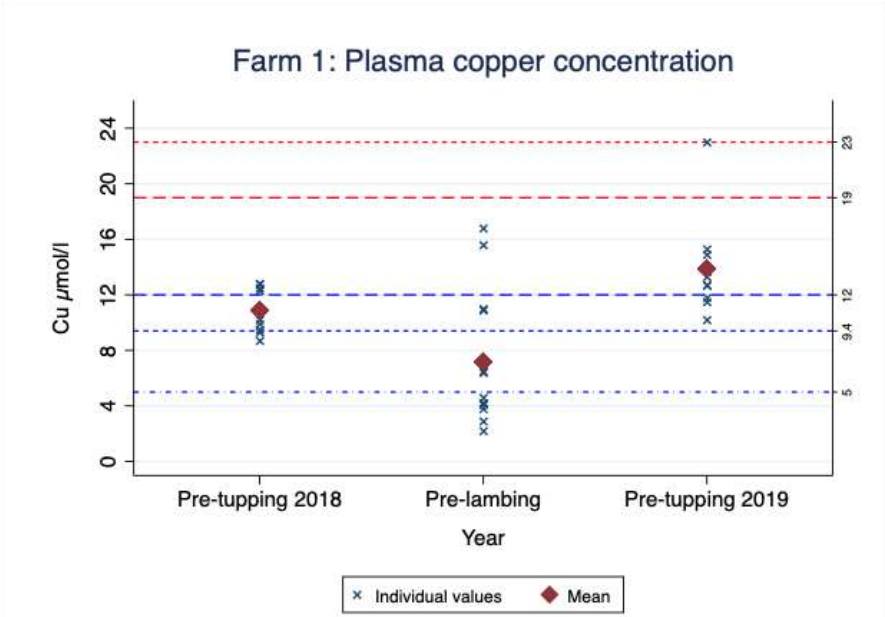
5. The old adages of ‘if you can’t measure it, you can’t manage it’ and ‘things are not always as they seem’ were frequently borne out throughout this project. As farms become larger and there is a move to focus on optimising production, close working relationships will need to continue to be developed between farmers and veterinary and consultancy services to enable farmers to achieve these goals.

Acknowledgements

I am grateful to valuable comments provided by both Gwyn Jones and Nigel Kendall to earlier drafts.

Appendix: Individual plots, by farm, for each analyte





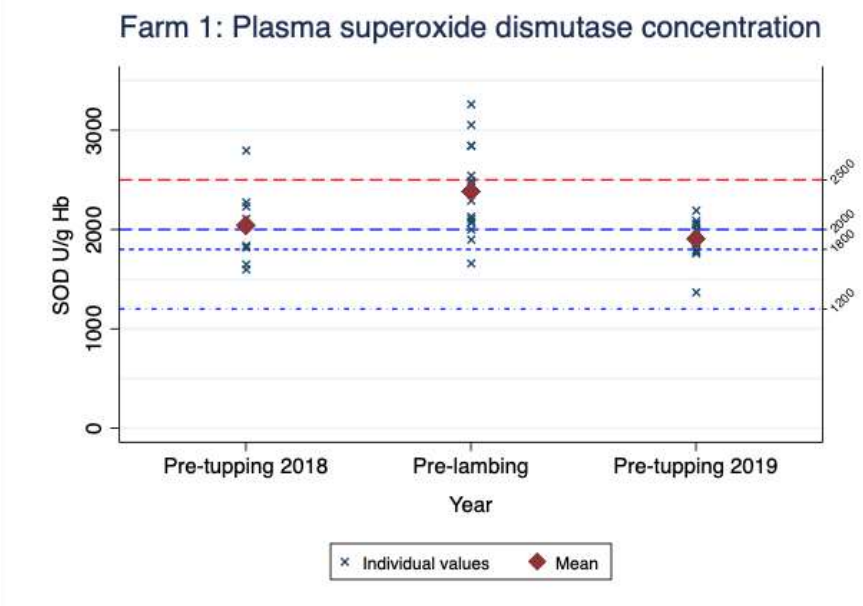
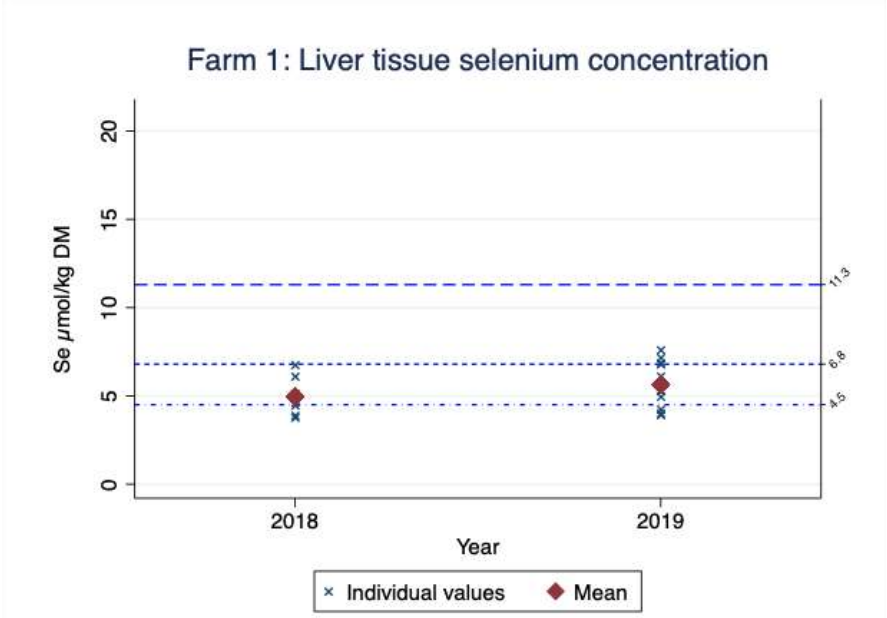
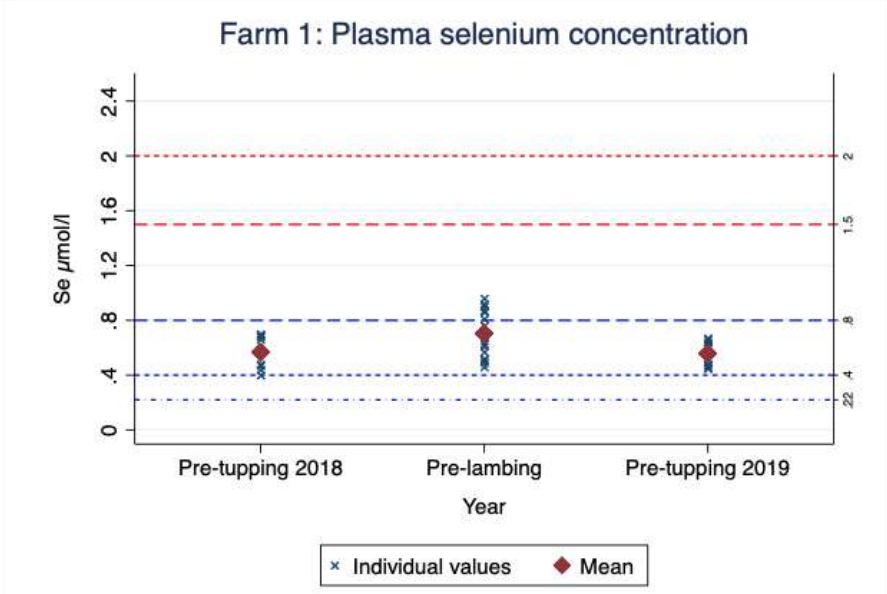
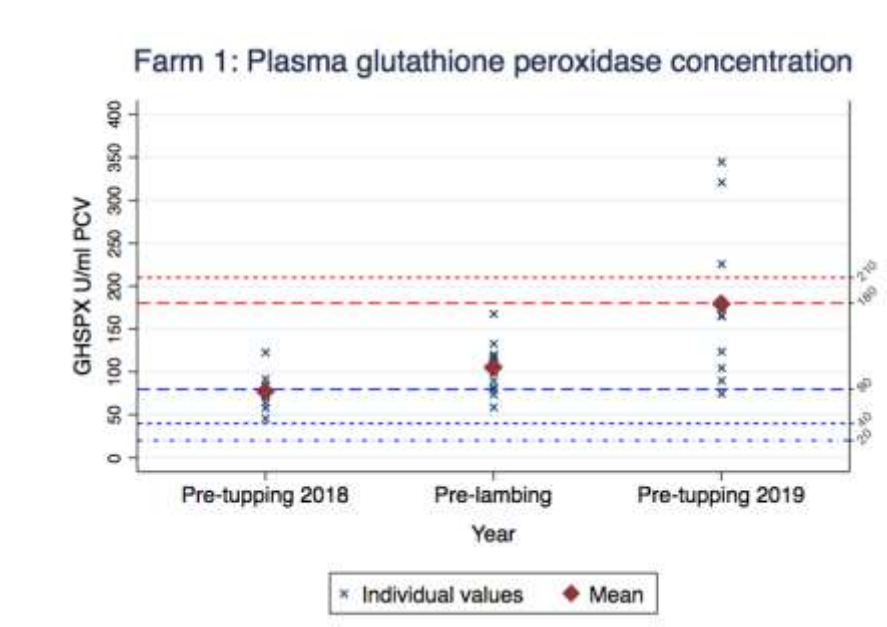


Figure 5: Copper analyses over time for Farm 1

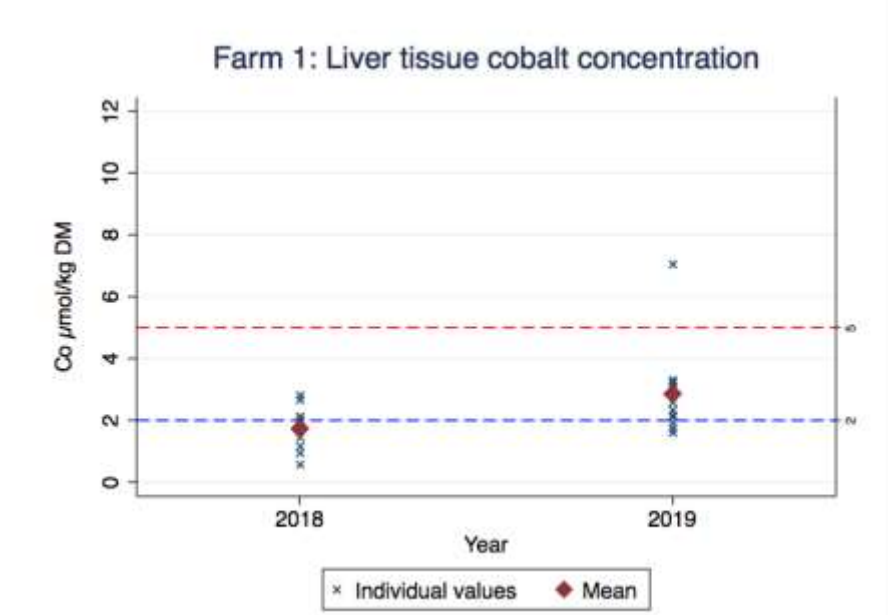

Farm 1: Liver tissue selenium concentration
Se µmol/kg DM
0
5
10
15
20
11.3
6.8
4.5
2018
2019
Year
Individual values
Mean

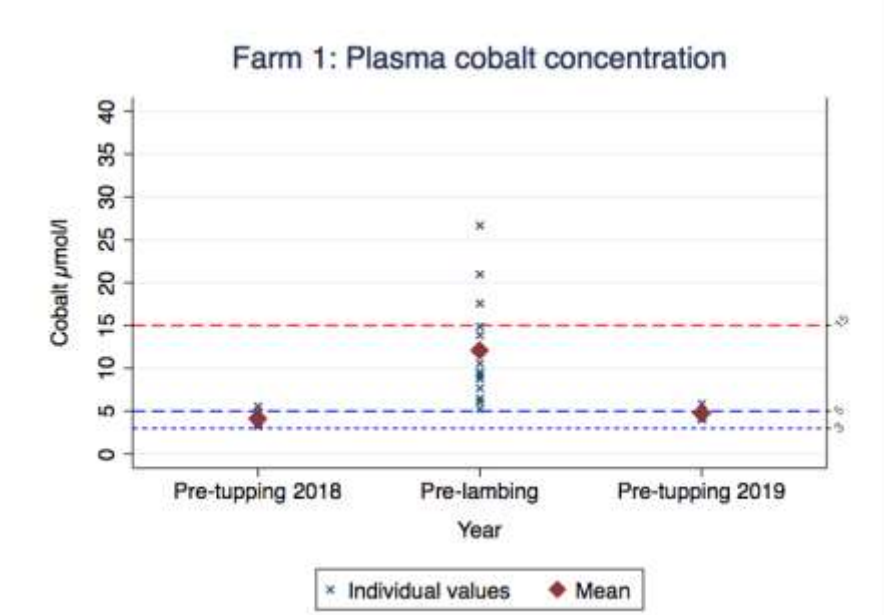

Farm 1: Plasma selenium concentration
Se µmol/l
0
.4
.8
1.2
1.6
2
2.4
2
1.5
.8
.4
.22
Pre-tupping 2018
Pre-lambing
Pre-tupping 2019
Year
Individual values
Mean

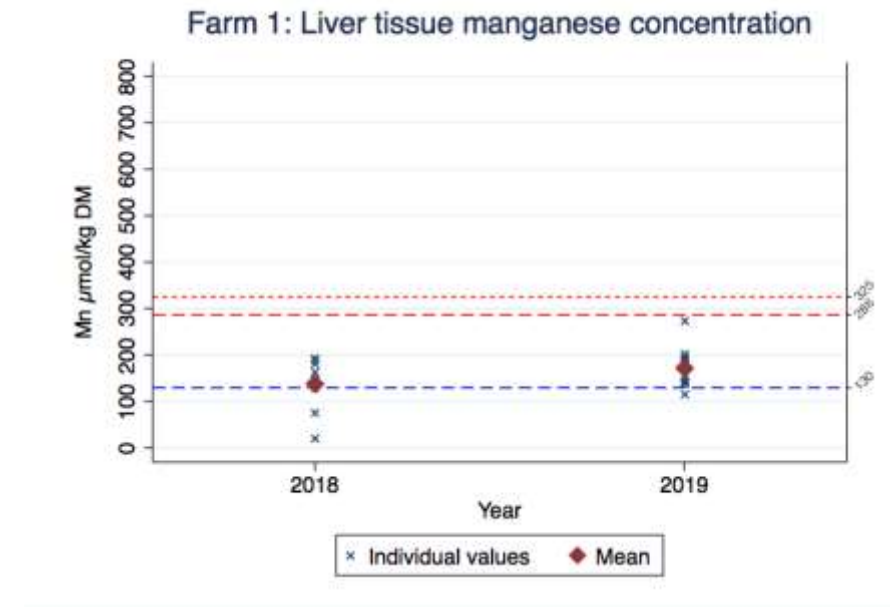

Farm 1: Plasma glutathione peroxidase concentration
GHSPX U/ml PCV
0
50
100
150
200
250
300
350
400
210
180
80
40
20
Pre-tupping 2018
Pre-lambing
Pre-tupping 2019
Year
Individual values
Mean

Figure

6: Selenium analyses over time for Farm 1







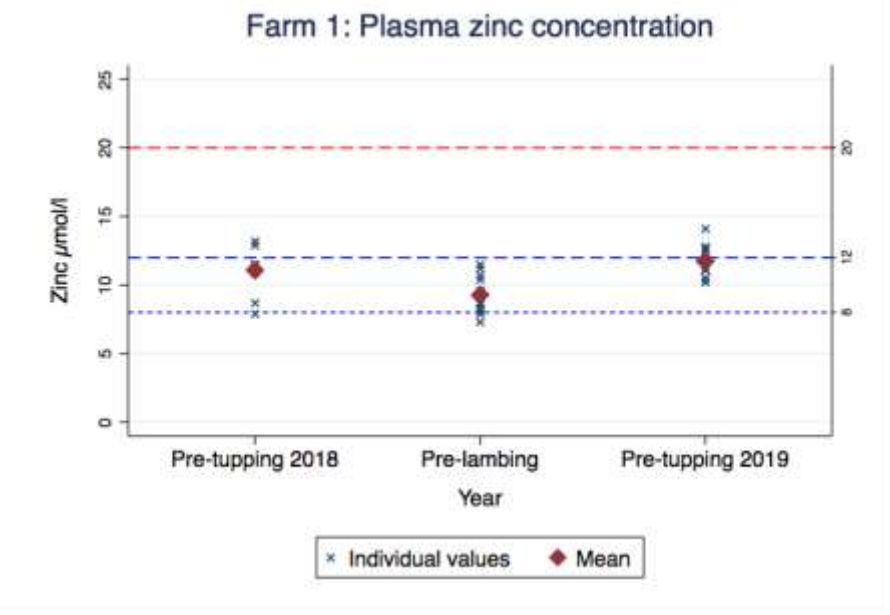
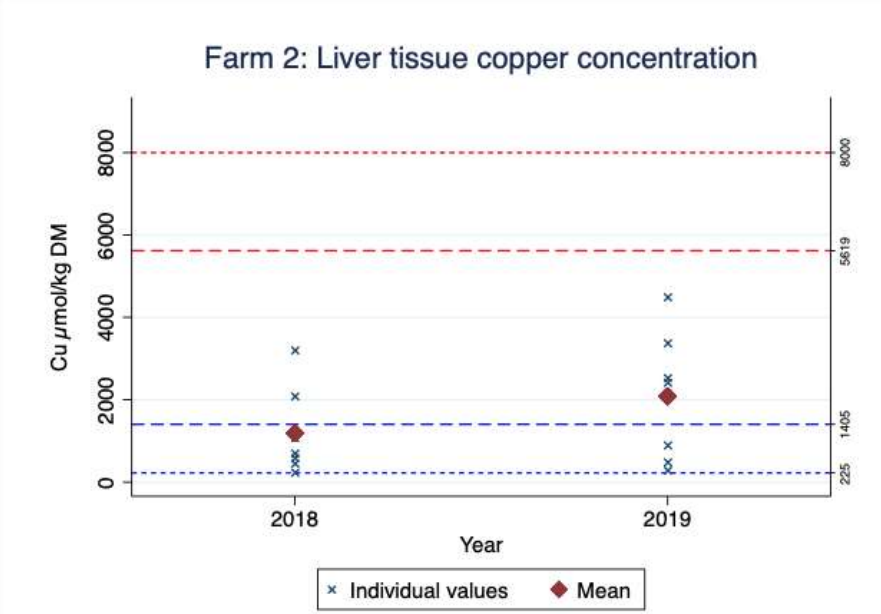
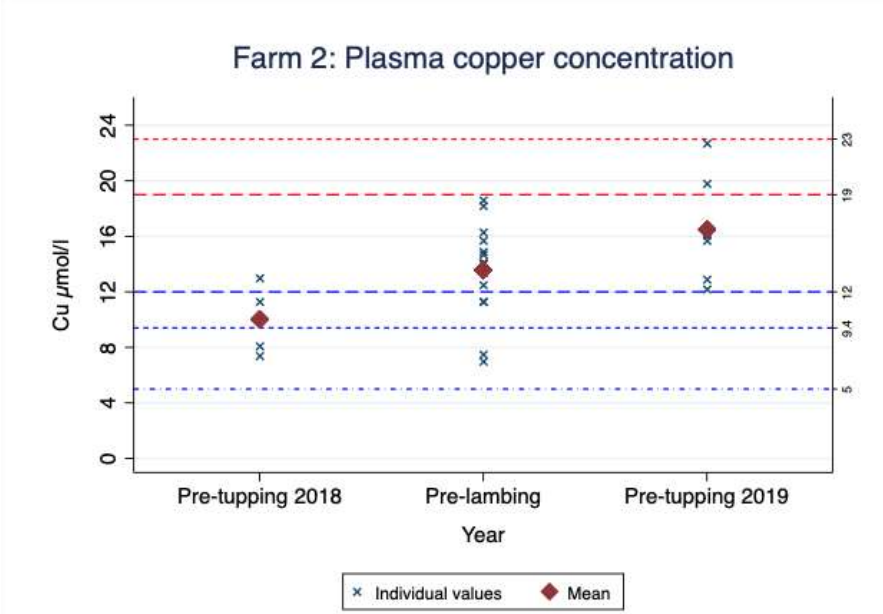
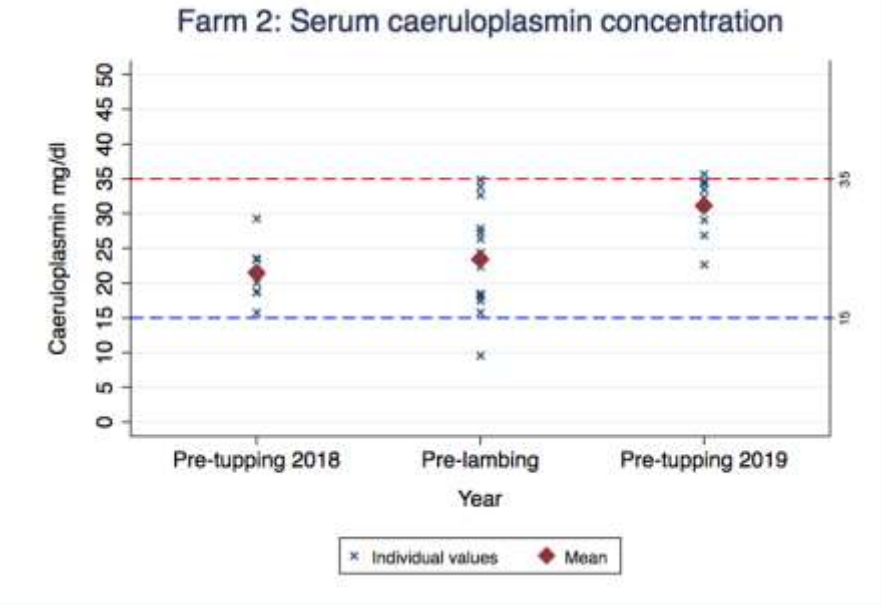


Figure 7: Cobalt, manganese and zinc analyses over time for Farm 1







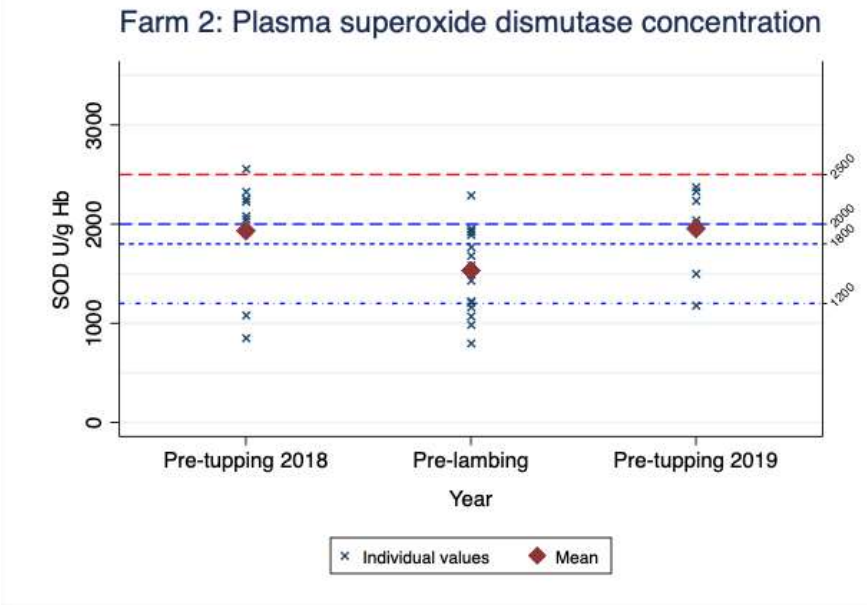


Figure 8: Copper analyses over time for Farm 2

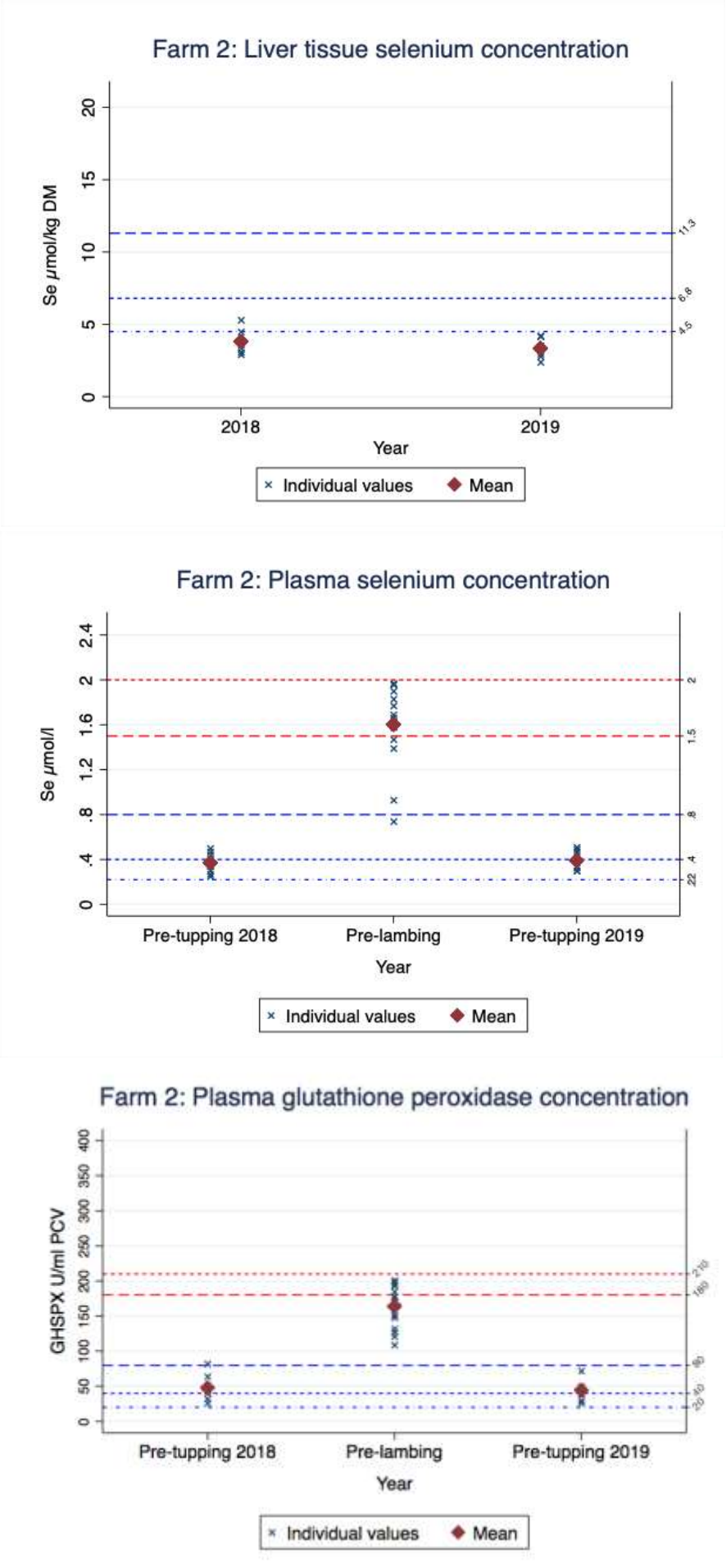
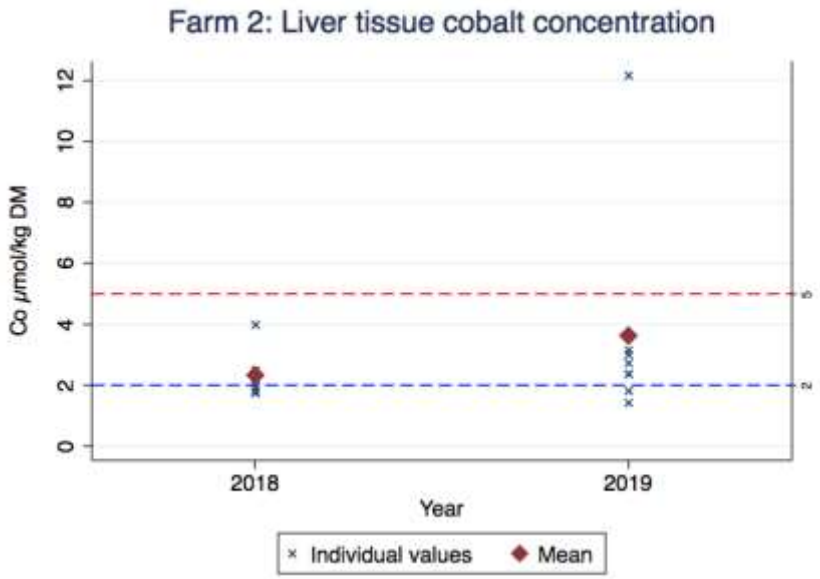
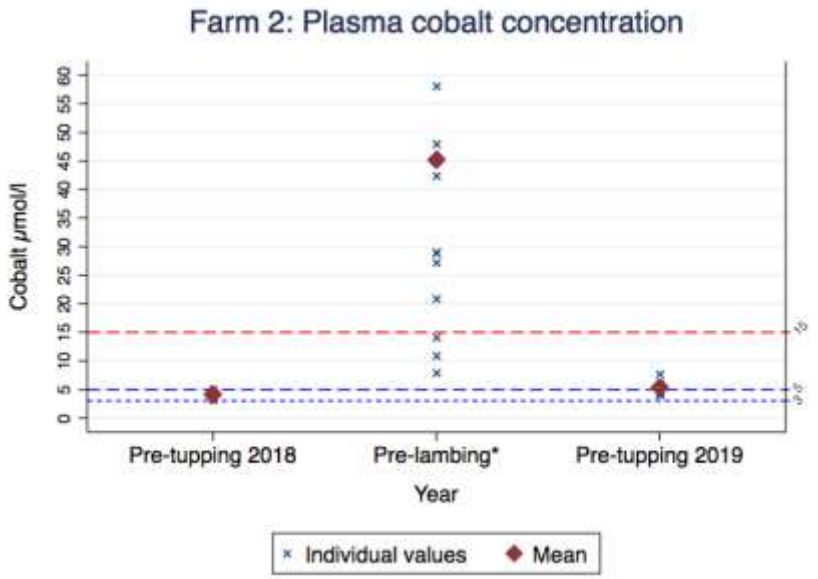
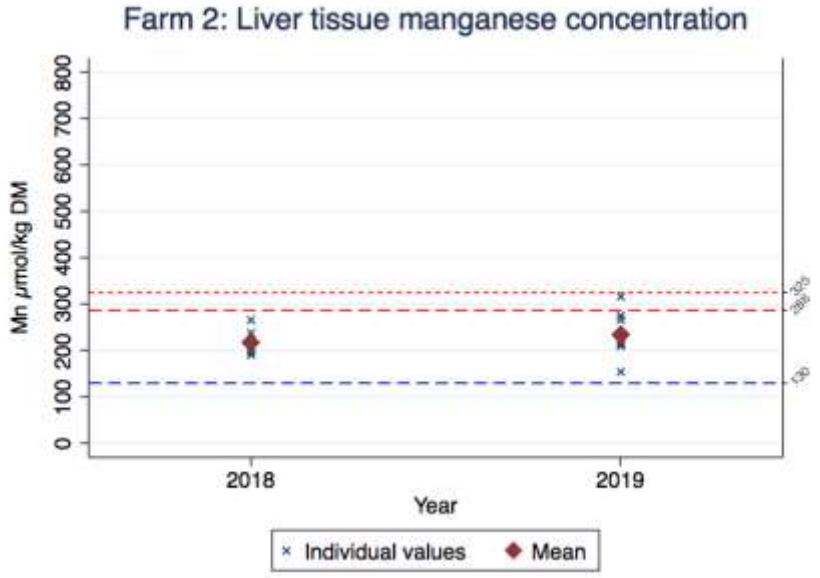


Figure 9: Selenium analyses over time for Farm 2







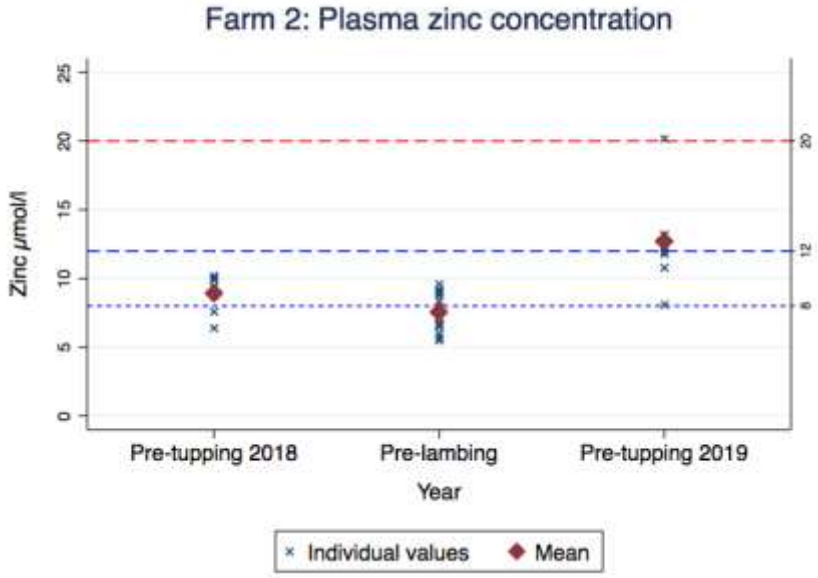
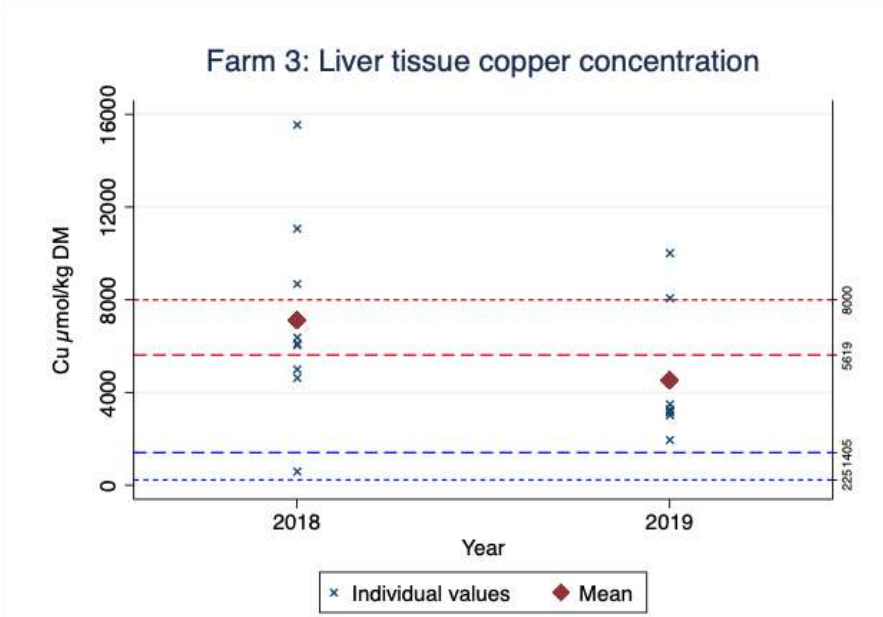
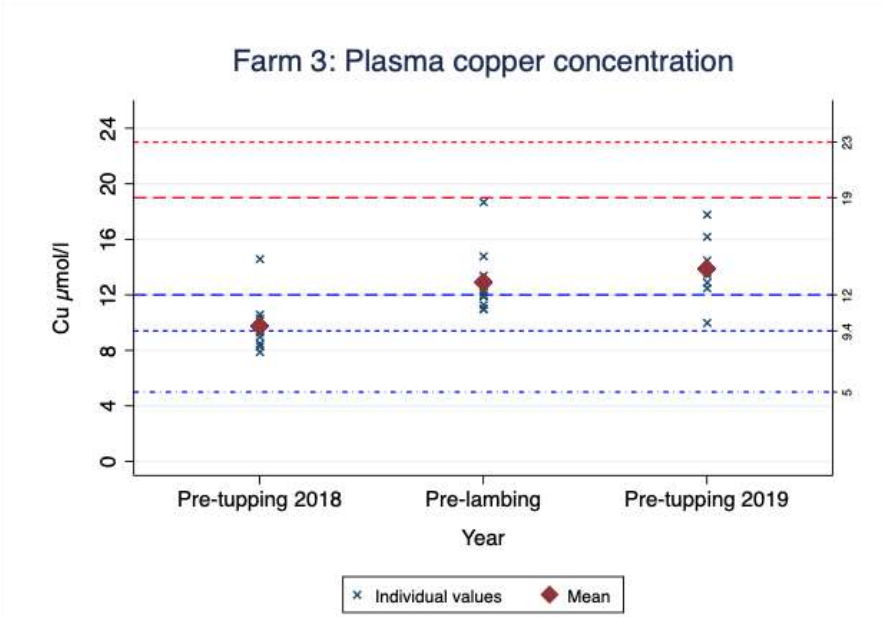
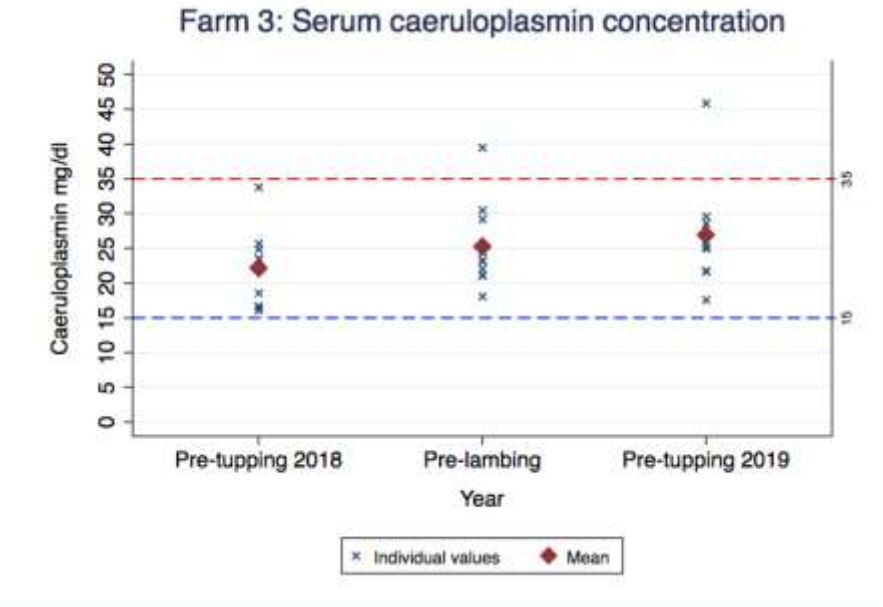


Figure 10: Cobalt, manganese and zinc analyses over time for Farm2; *in the interests of clarity, some very high values in the pre-lambing samples have been omitted.







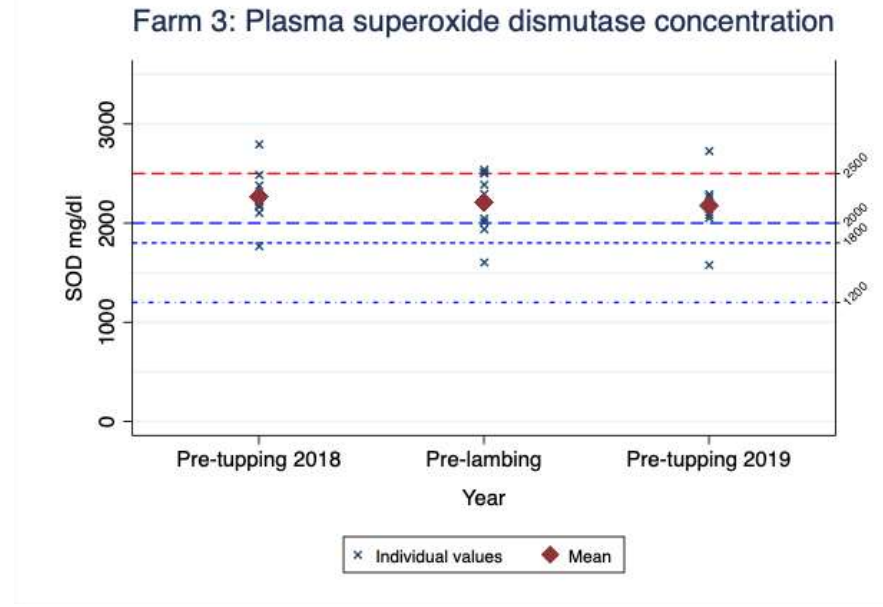


Figure 11: Copper analyses over time for Farm 3

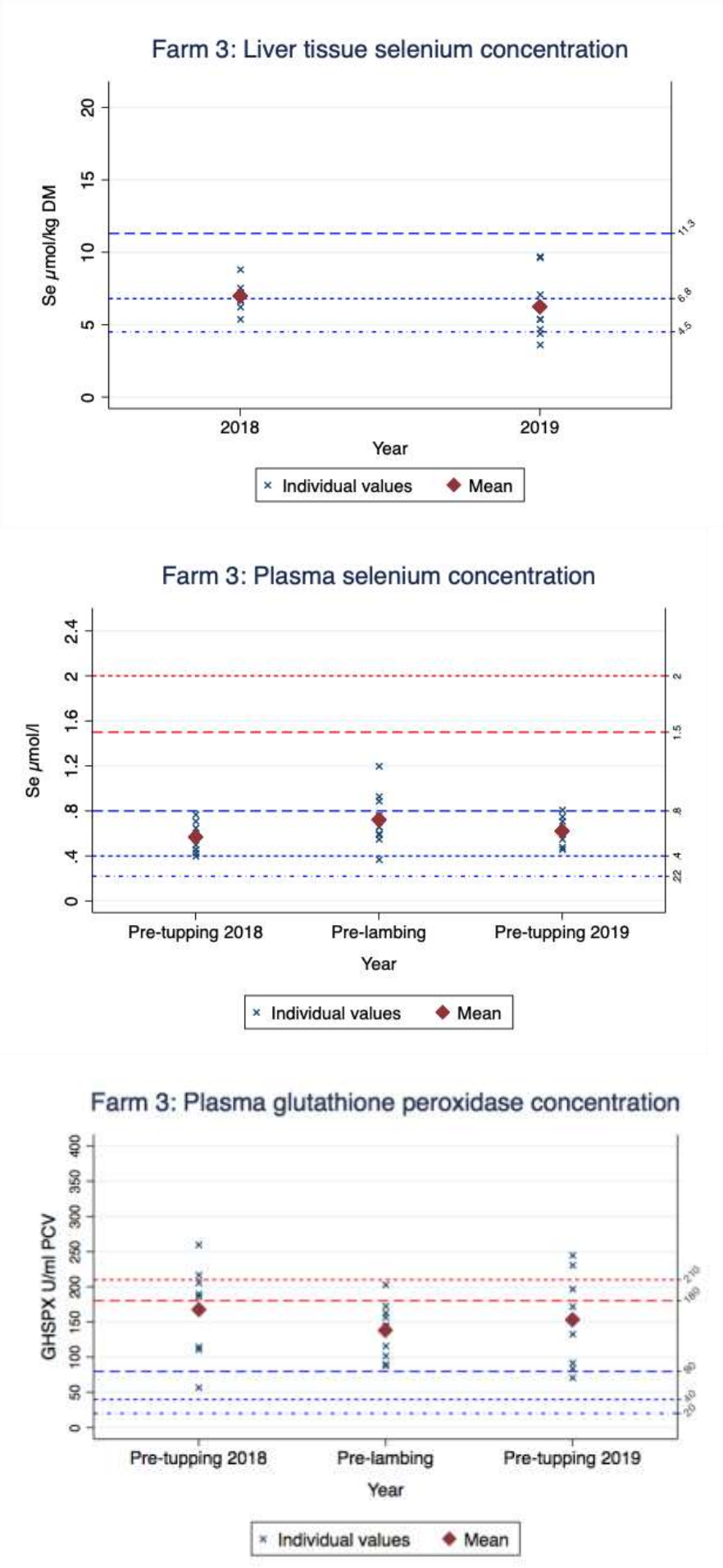


Figure 12: Selenium analyses over time for Farm 3

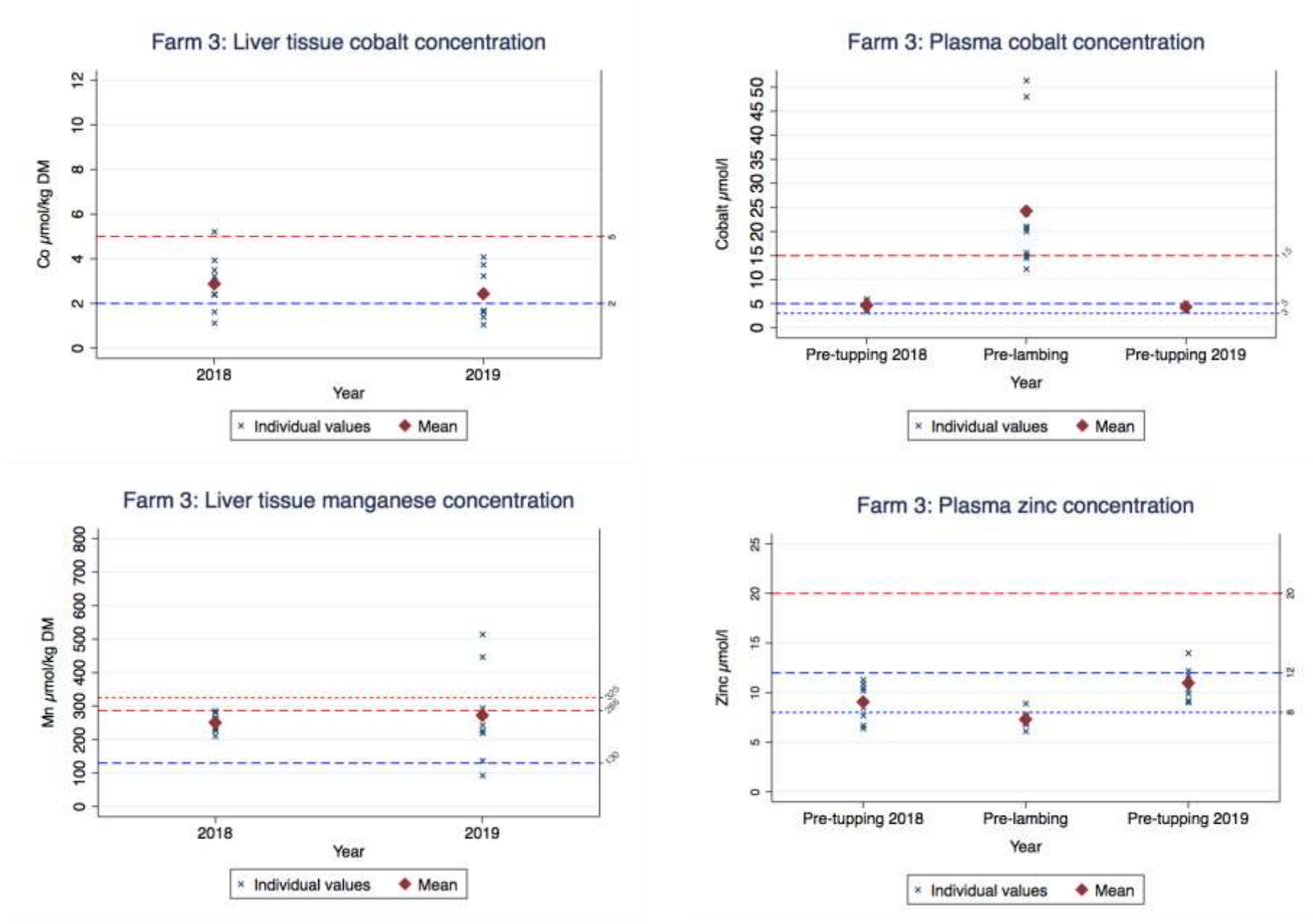


Figure 13: Cobalt, manganese and zinc analyses over time for Farm 3

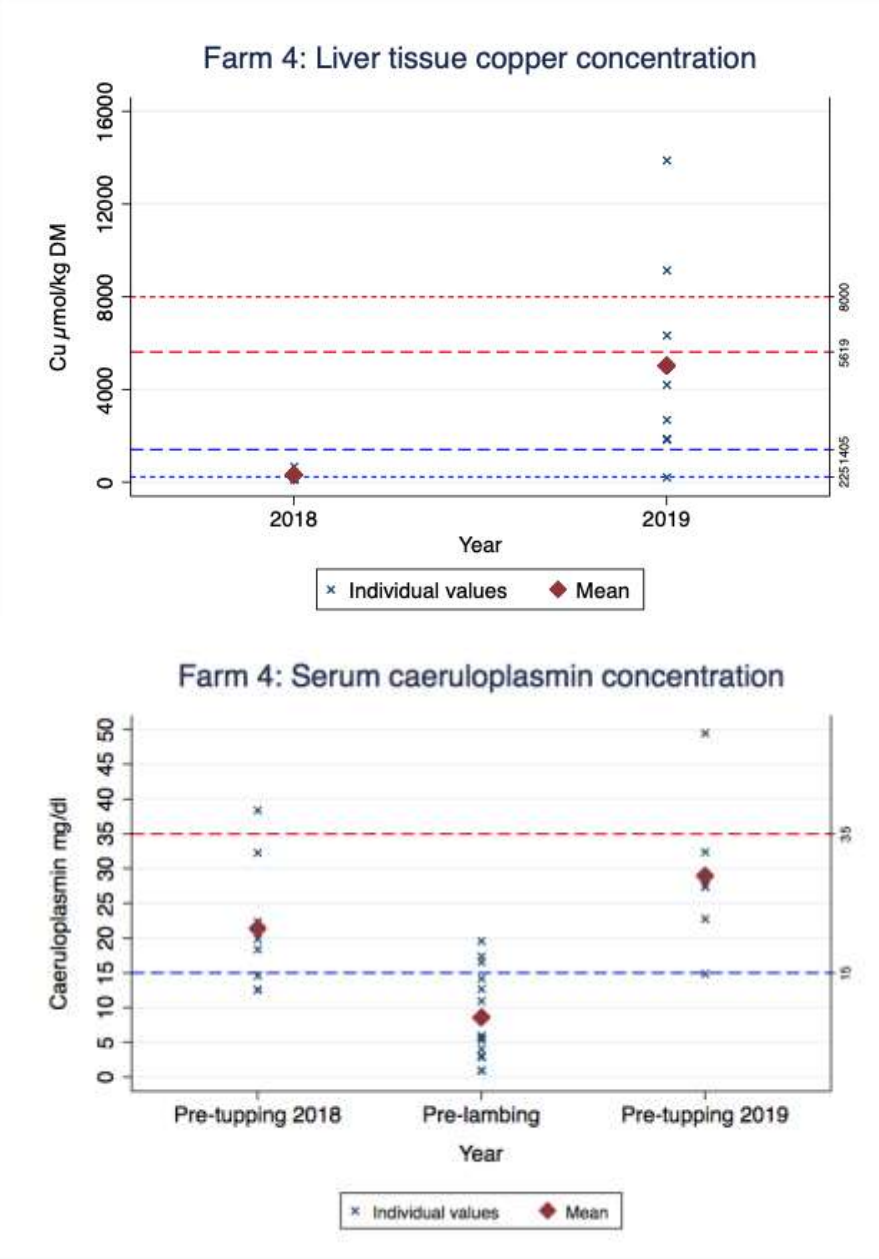
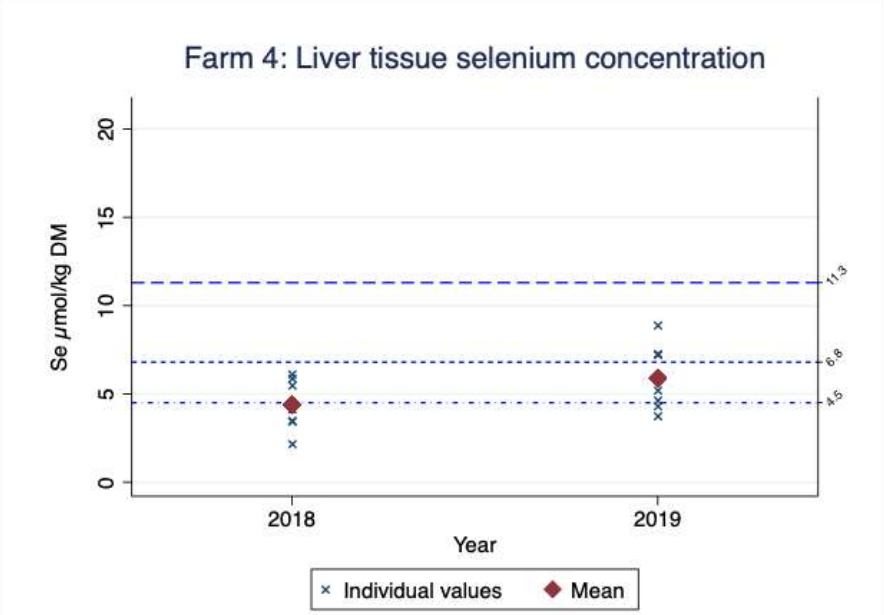
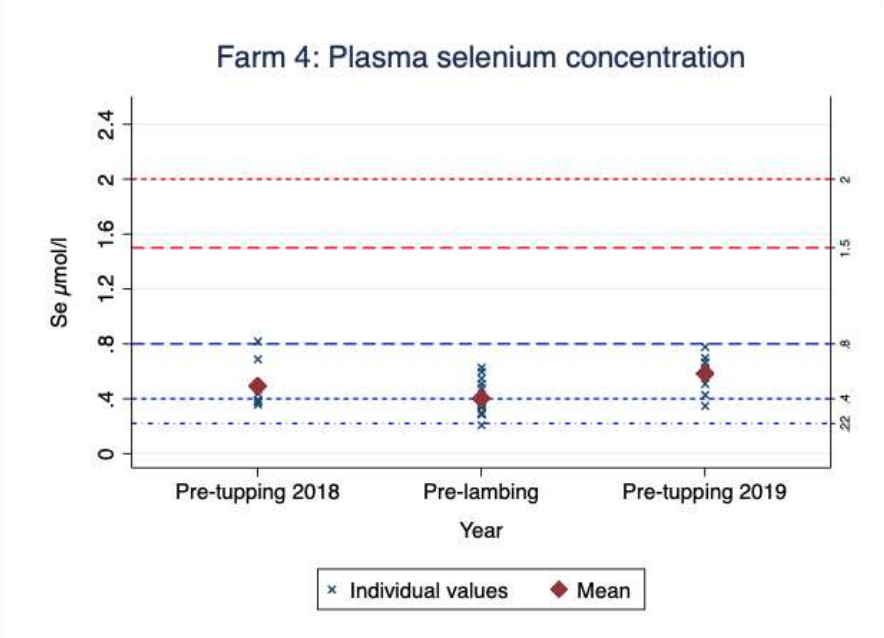


Figure 14: Copper analyses over time for Farm 4





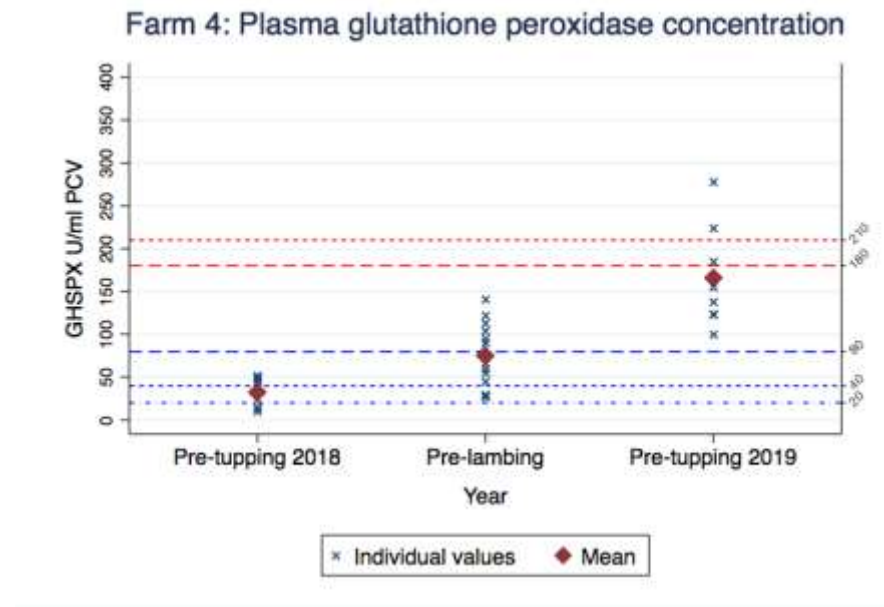
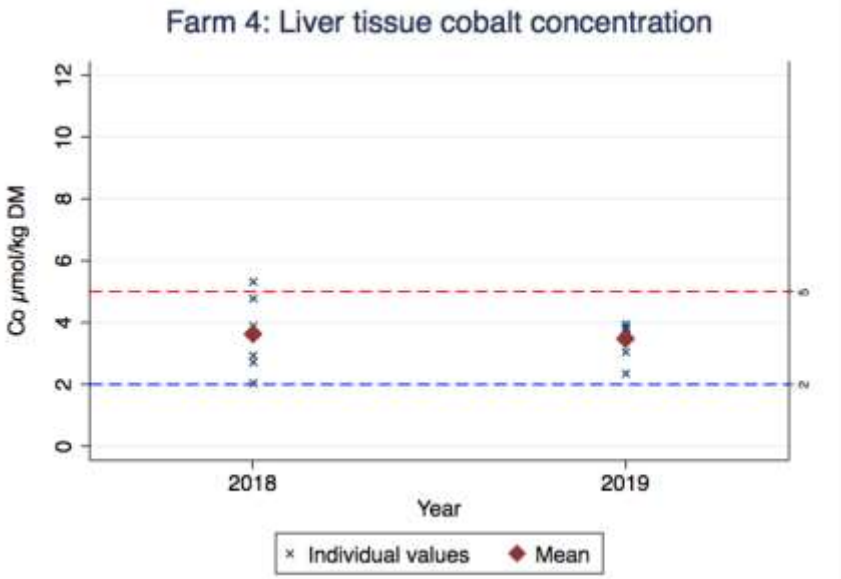
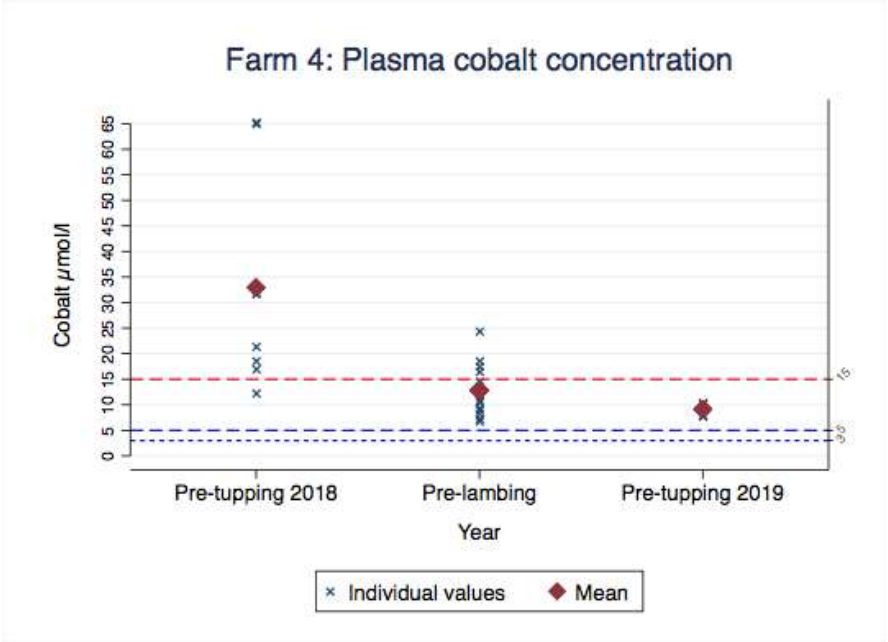
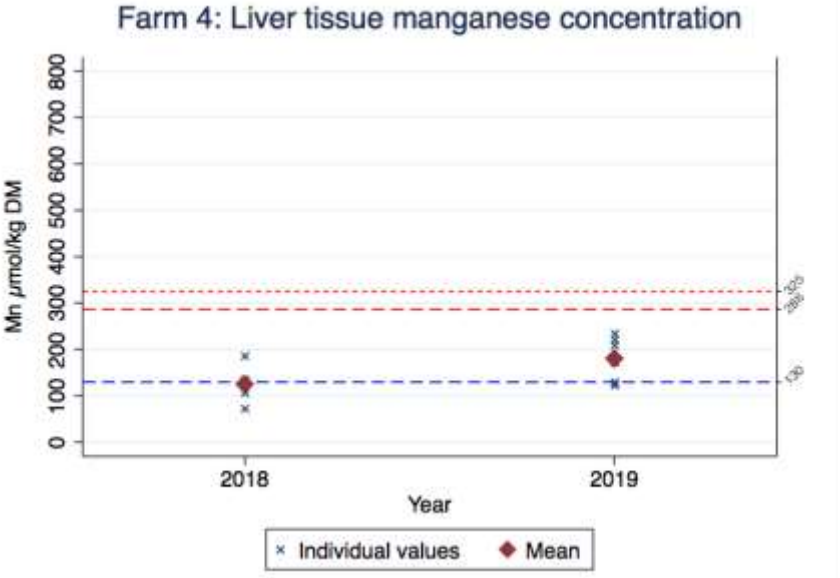


Figure 15: Selenium analyses over time for Farm 4







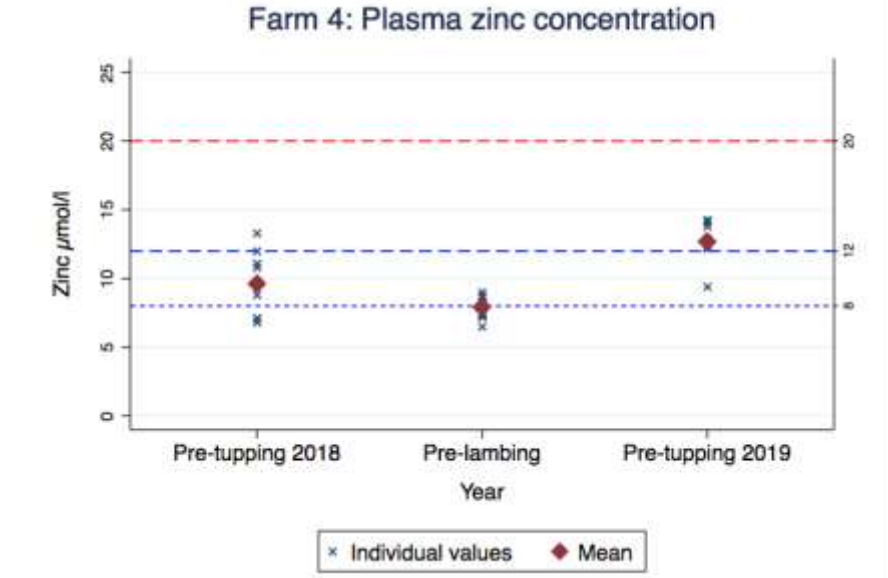
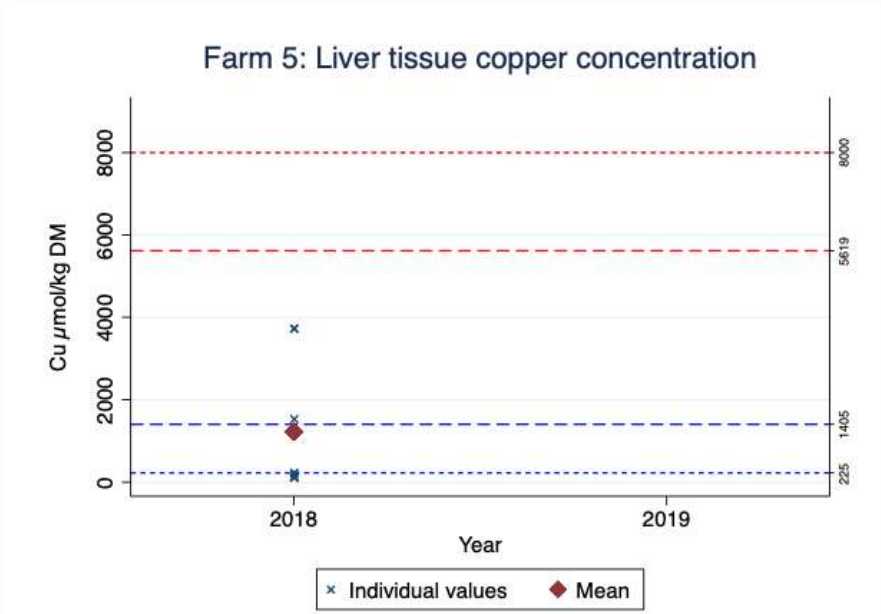
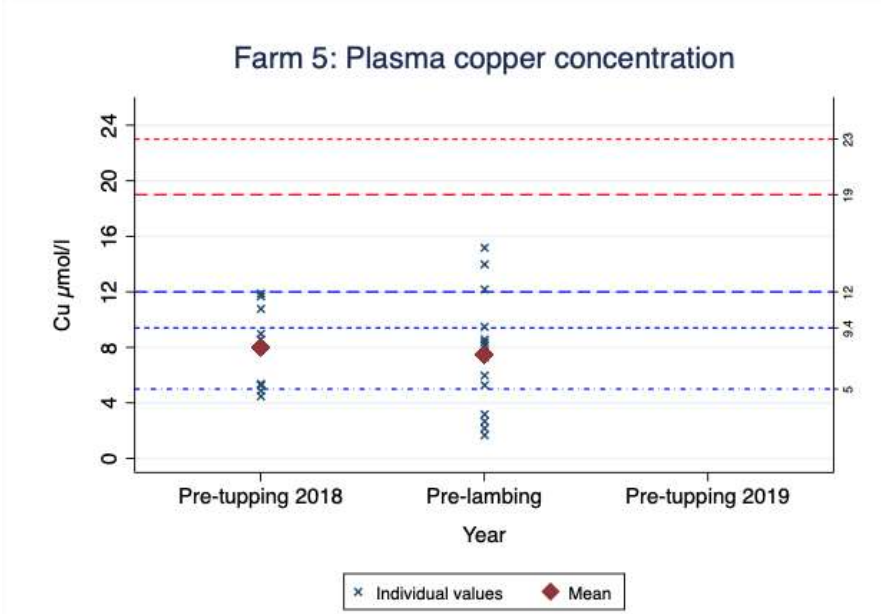
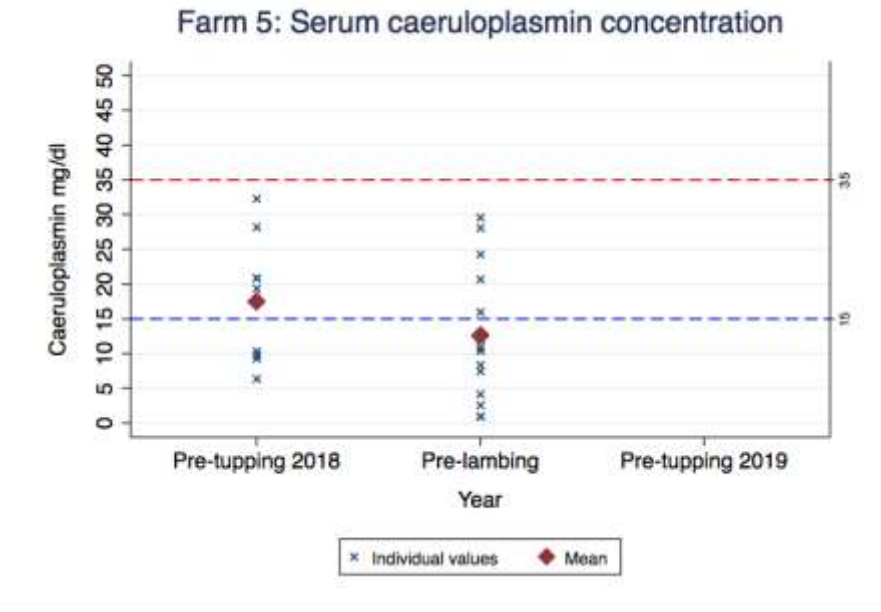


Figure 16: Cobalt, manganese and zinc analyses over time for Farm 4







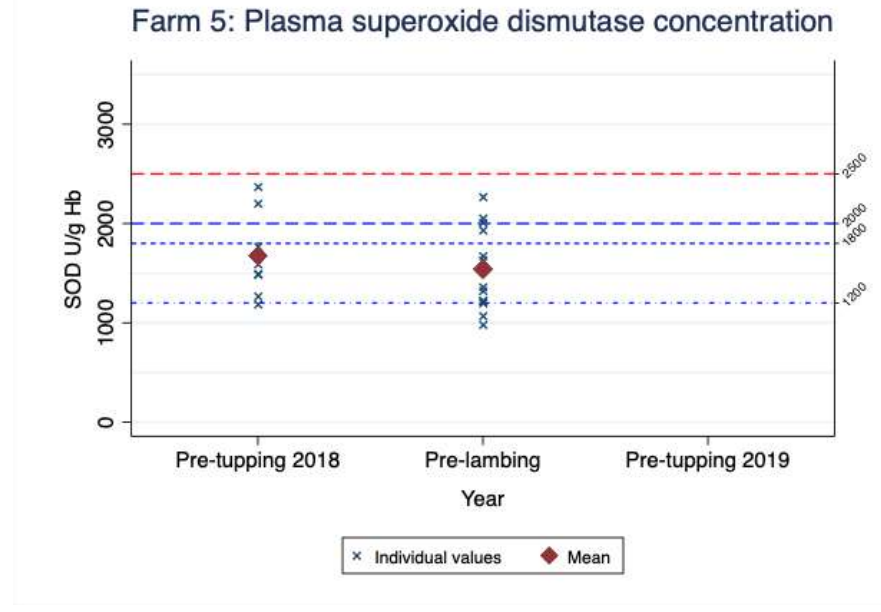
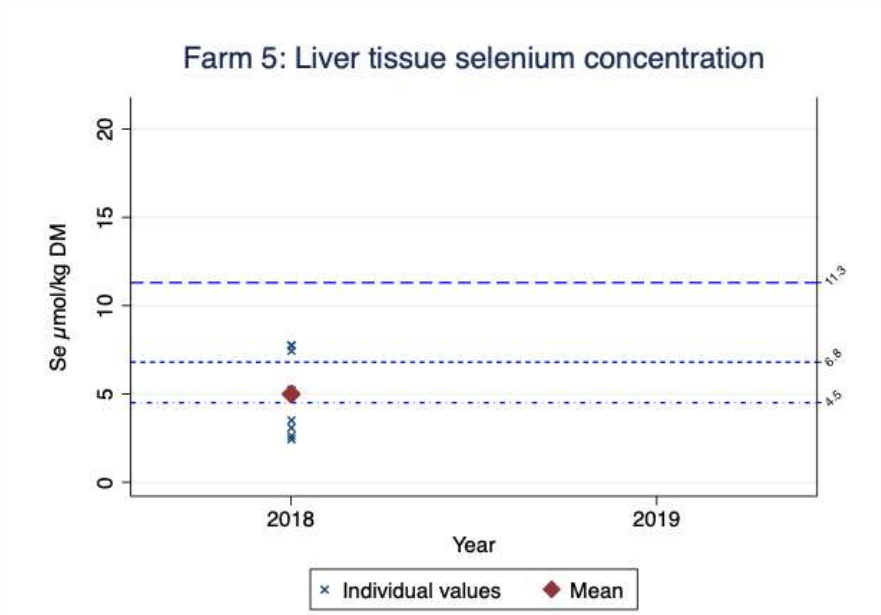
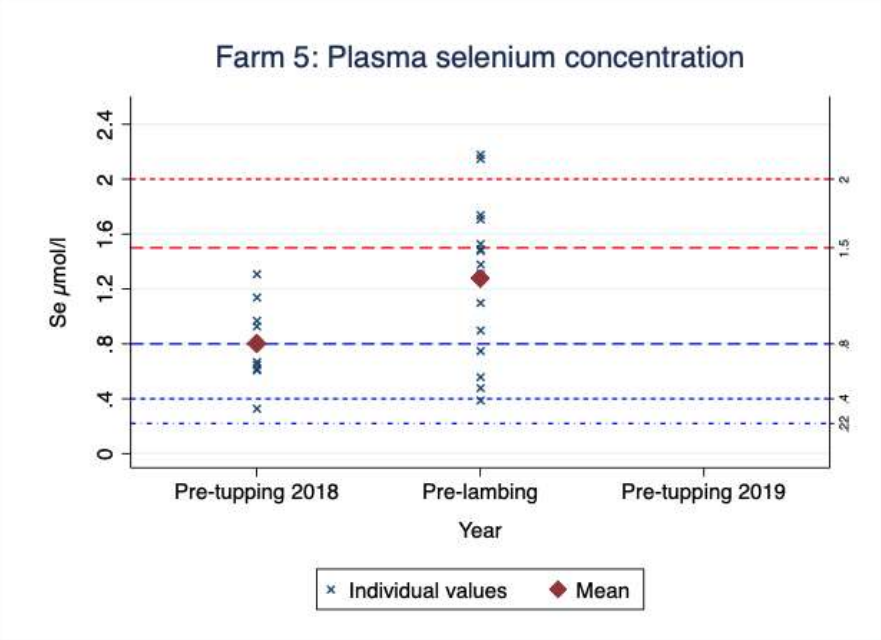


Figure 17: Copper analyses over time for Farm 5





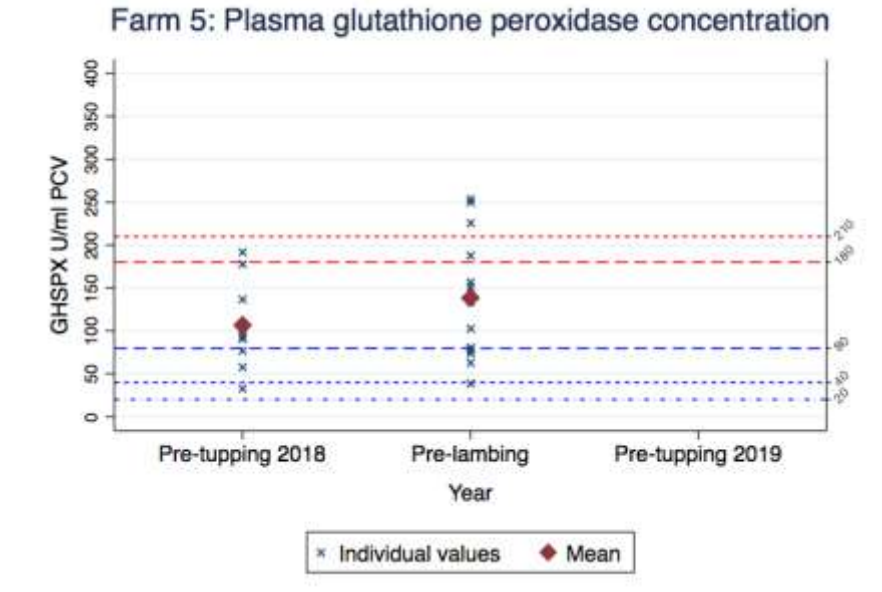


Figure 18: Selenium analyses over time for Farm 5

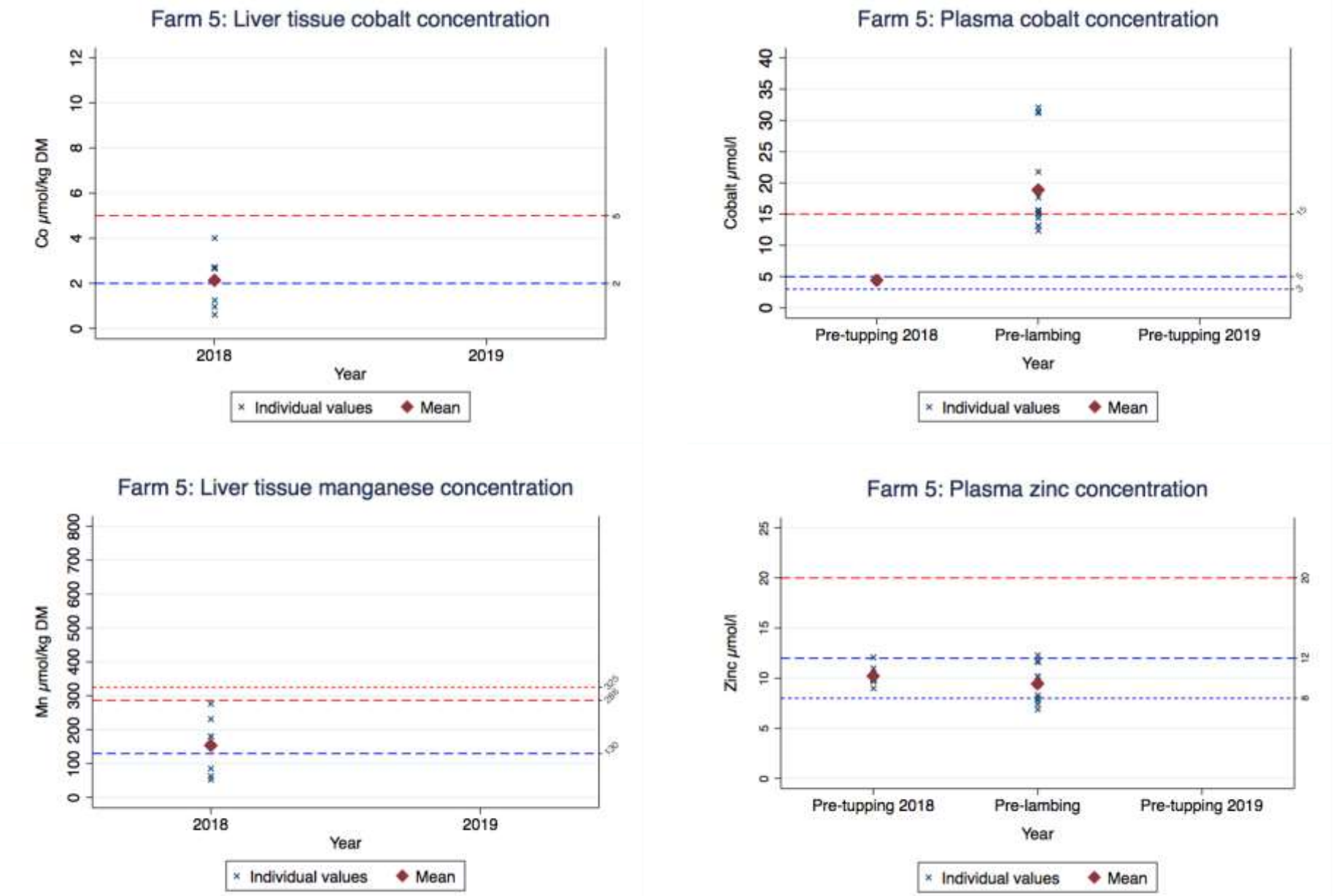
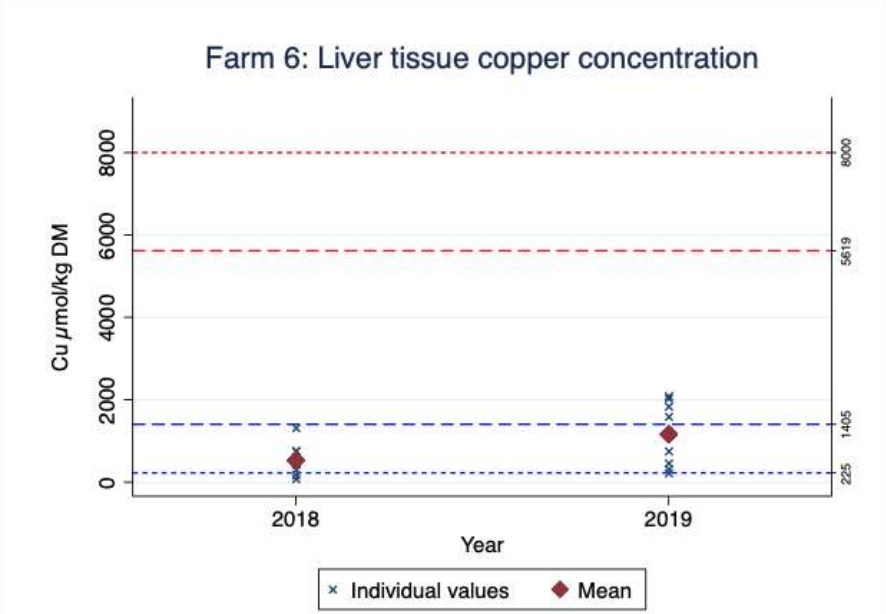
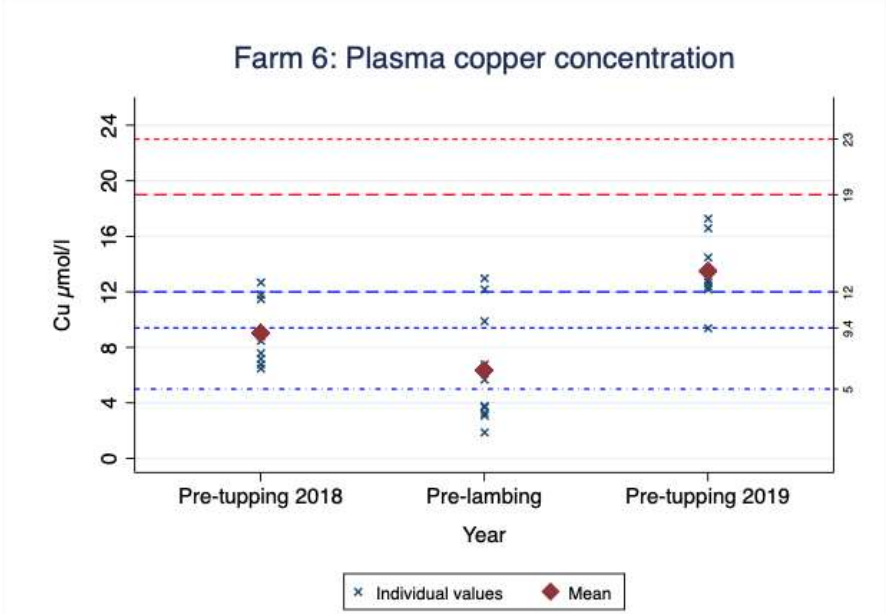
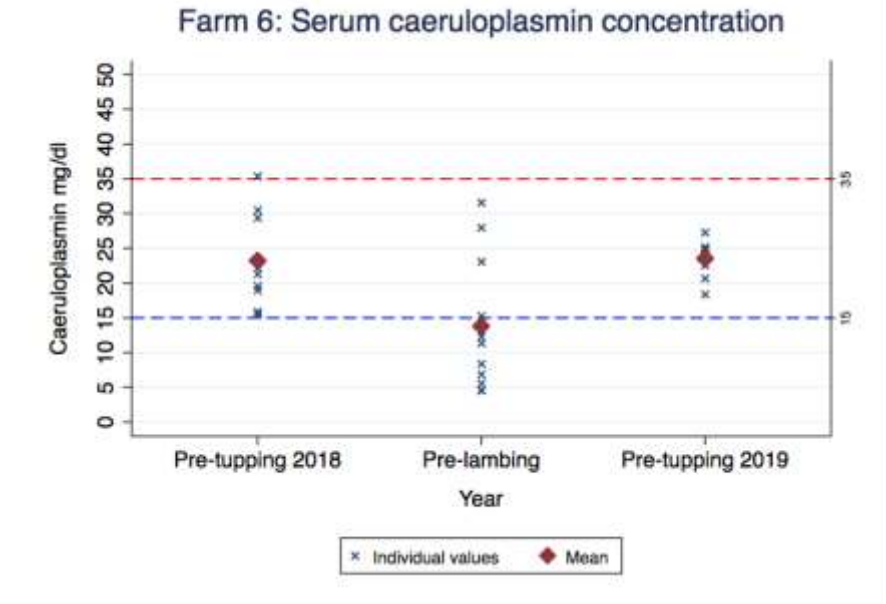


Figure 19: Cobalt, manganese and zinc analyses over time for Farm 5







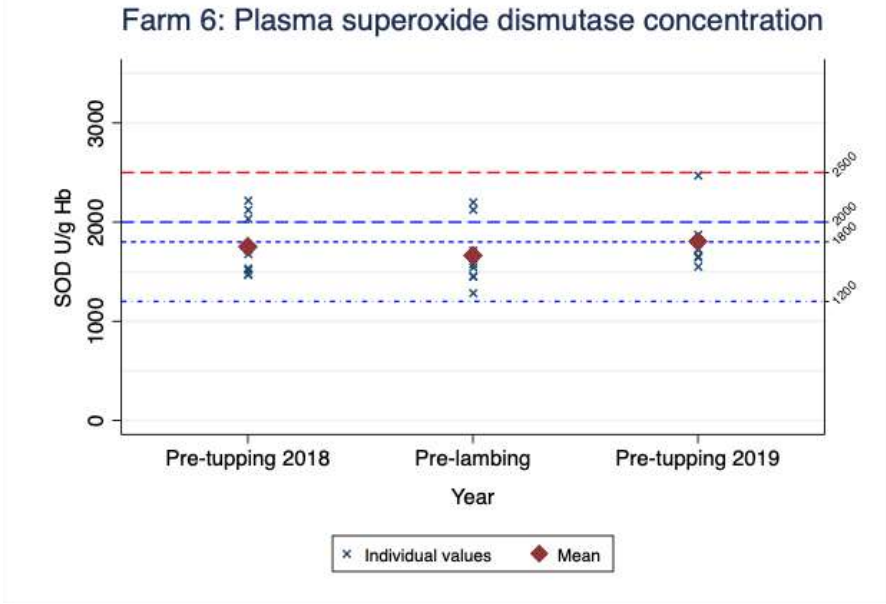
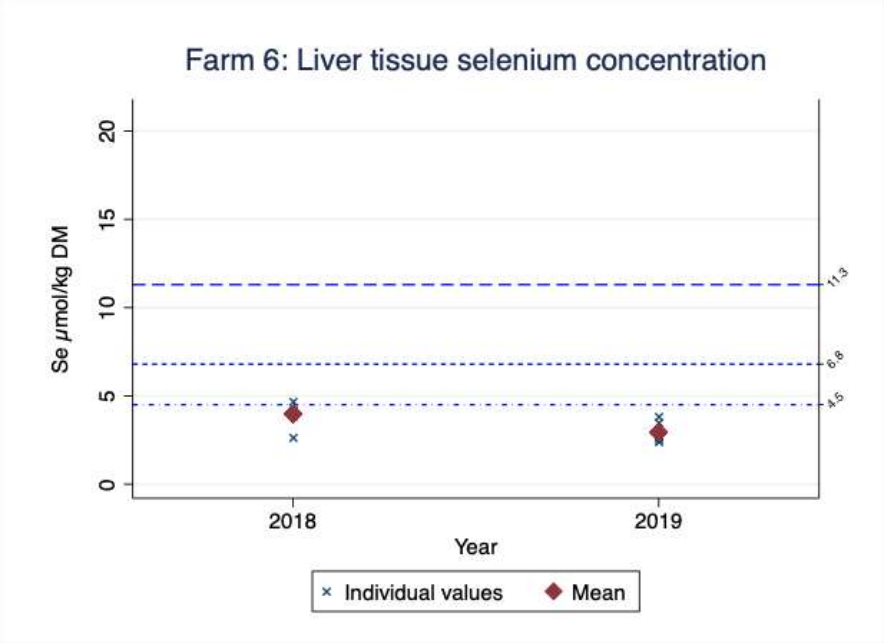
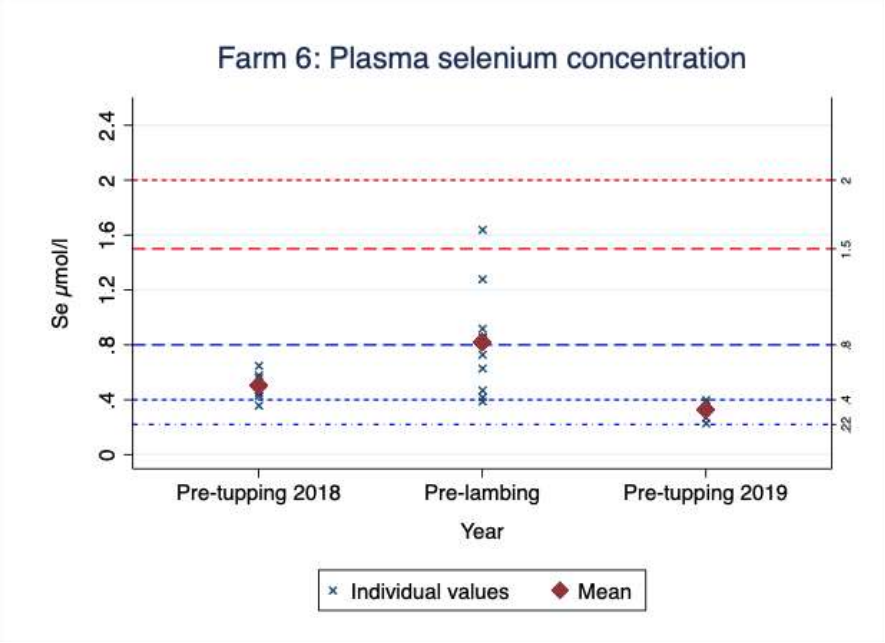


Figure 20: Copper analyses over time for Farm 6





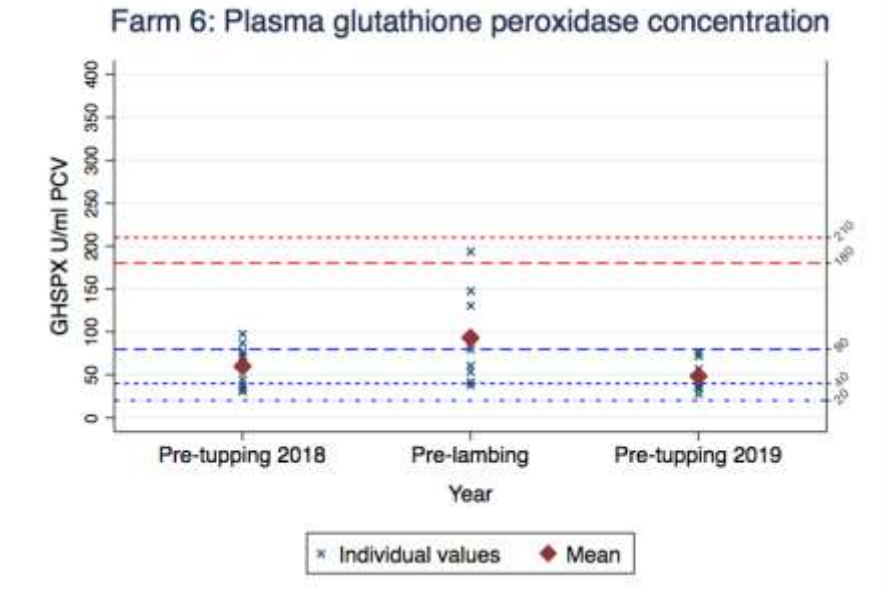
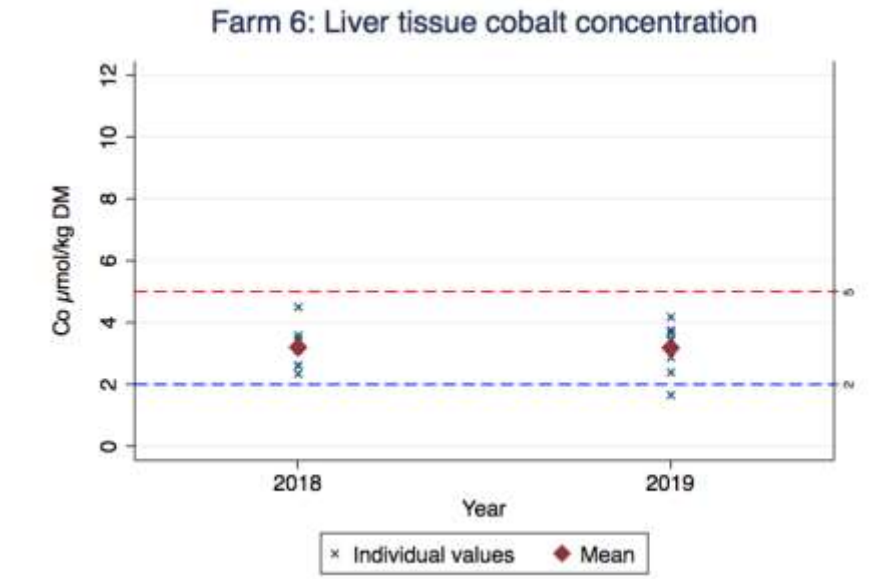
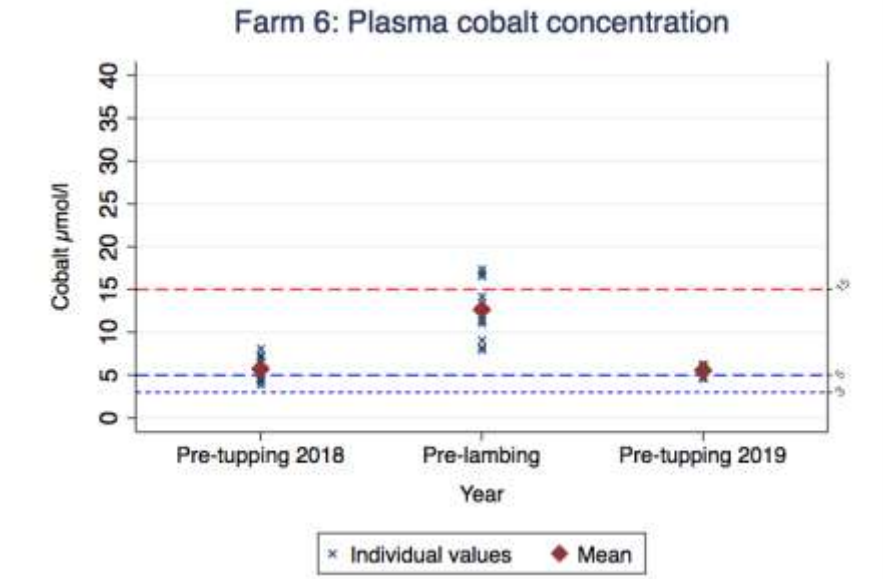
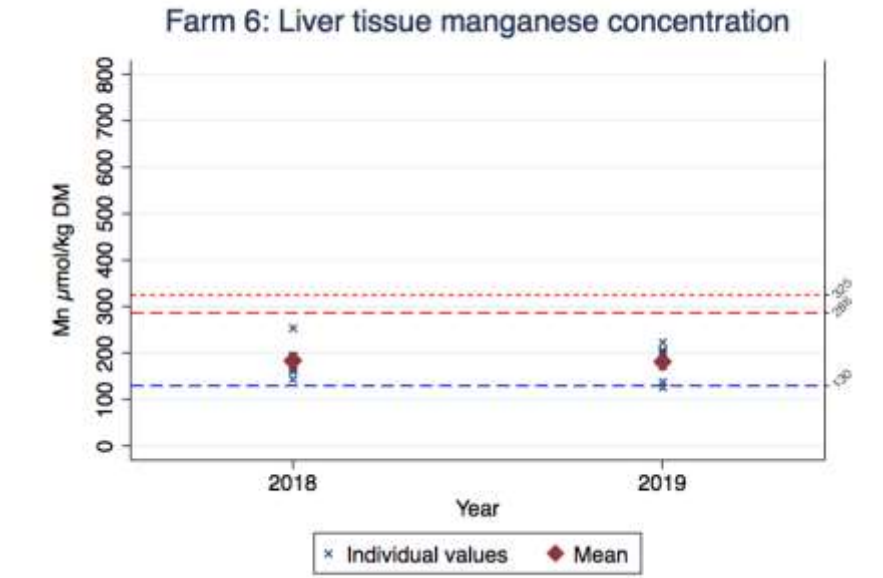


Figure 21: Selenium analyses over time for Farm 6







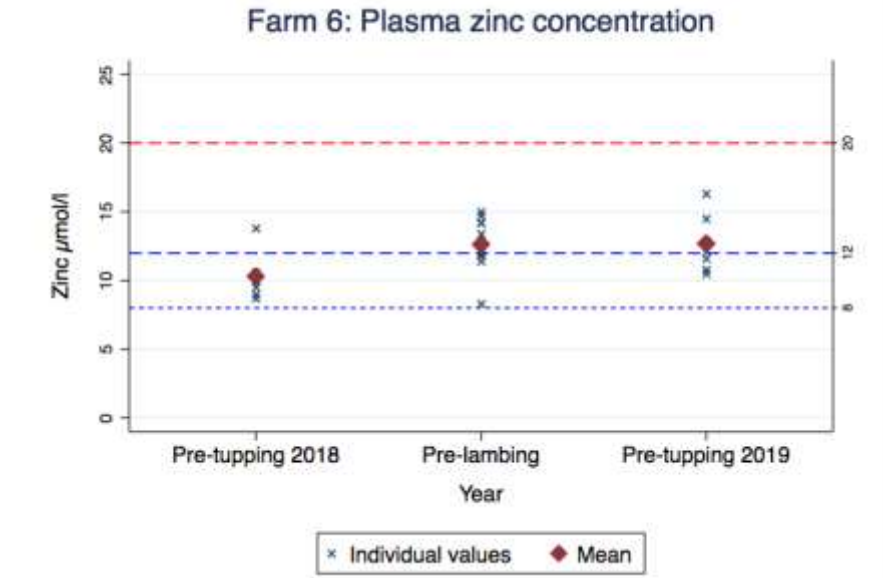
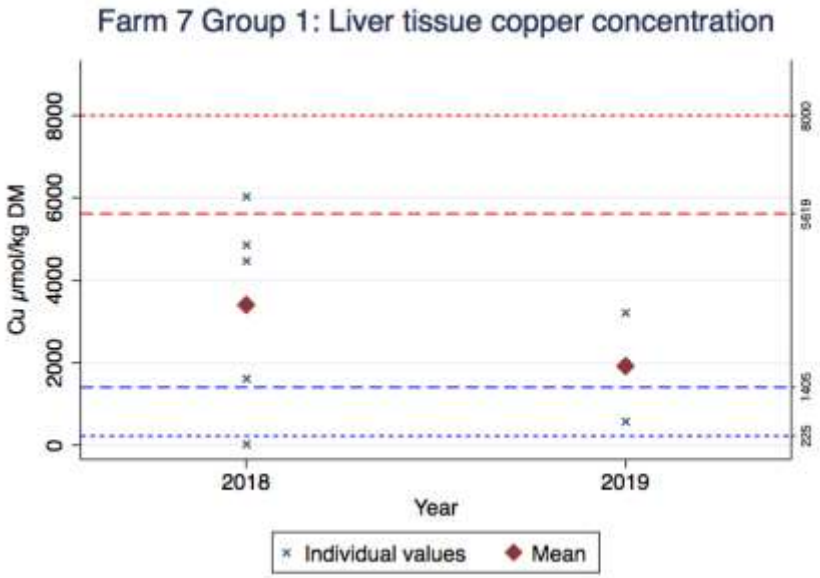
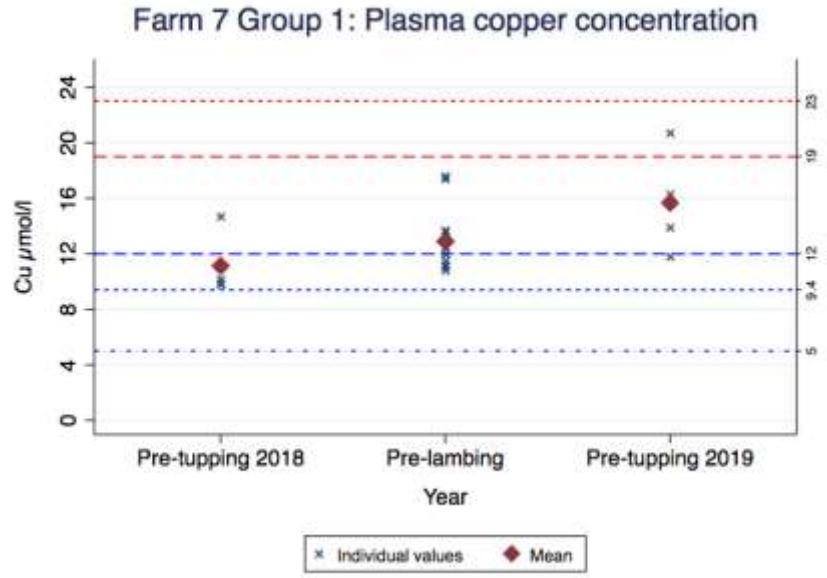
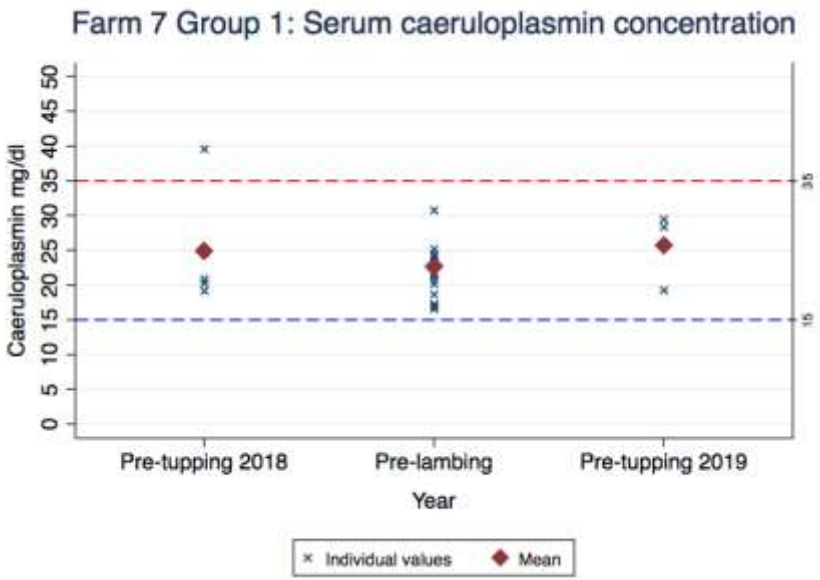


Figure 22: Cobalt, manganese and zinc analyses over time for Farm 6







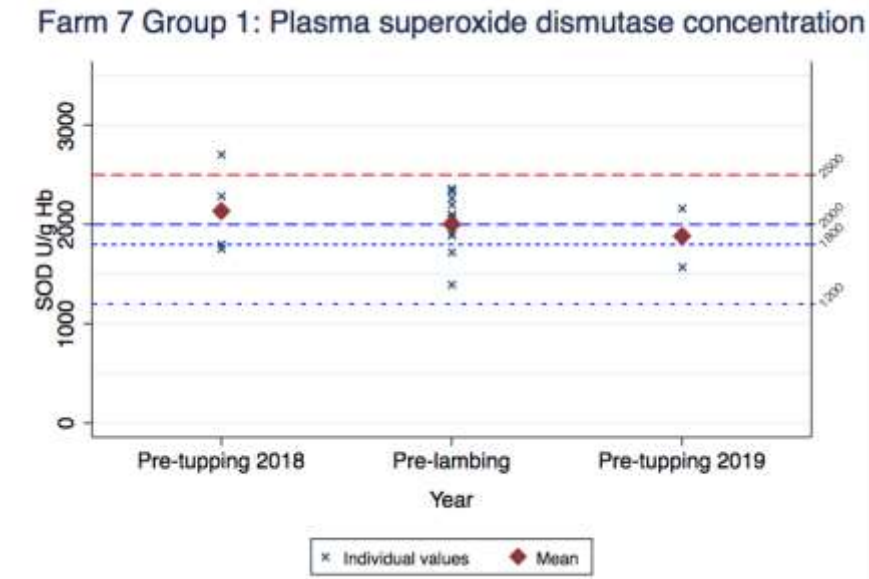
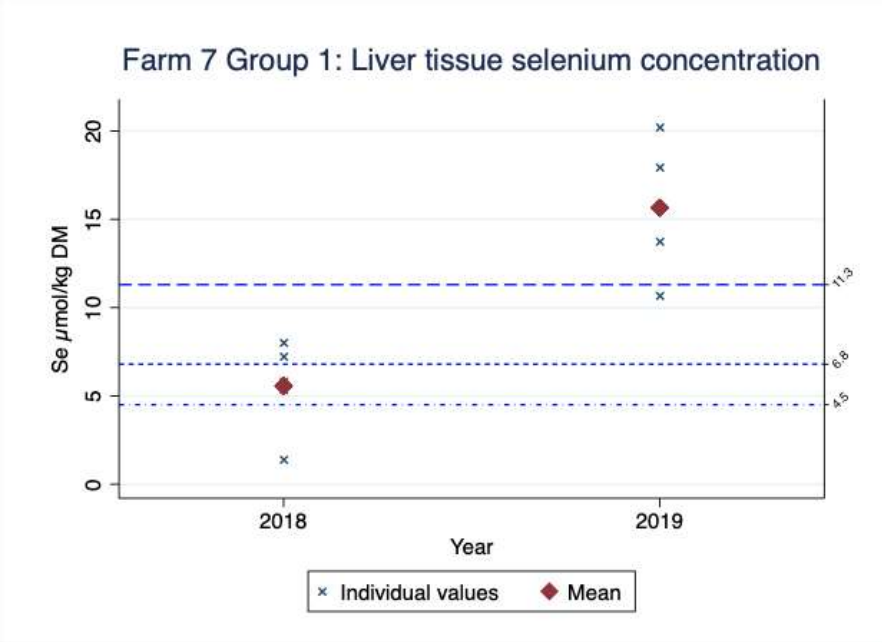
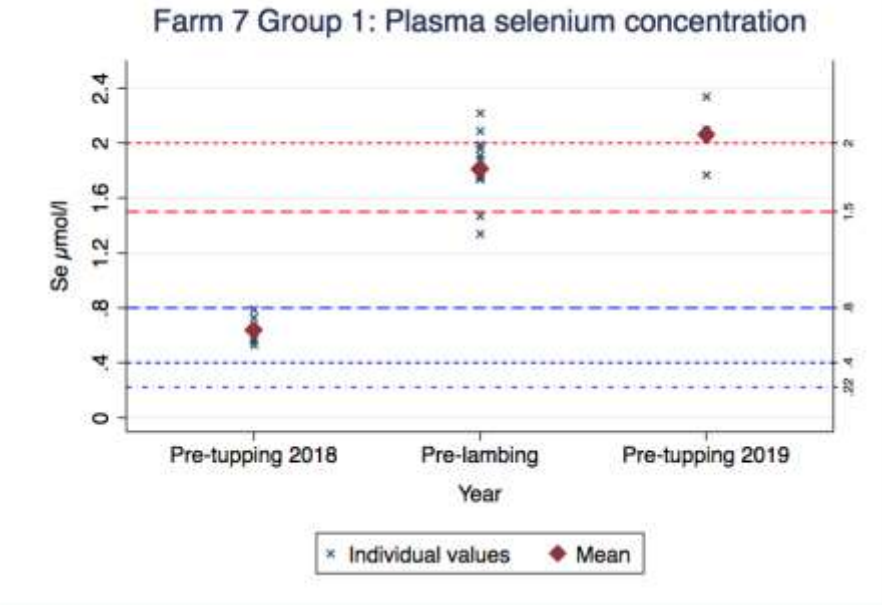


Figure 23: Copper analyses over time for Farm 7, Group 1





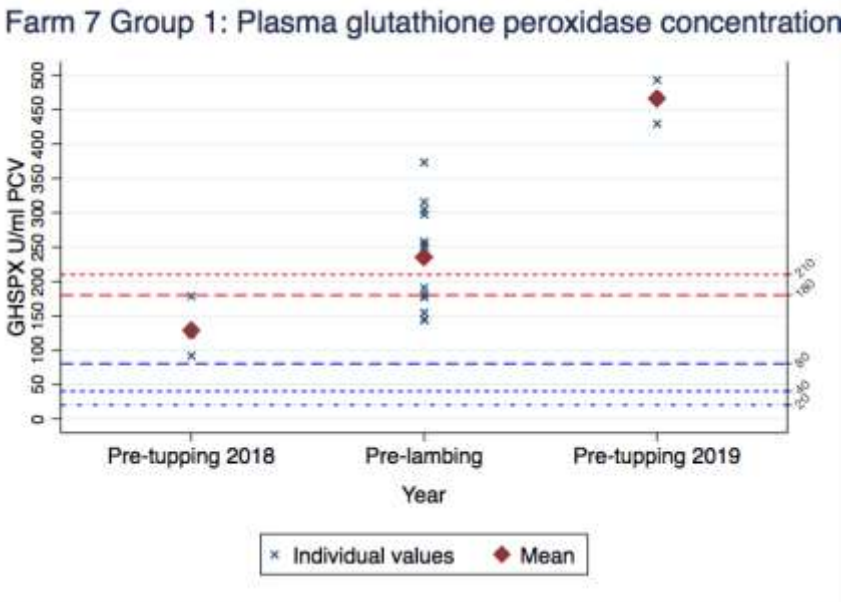


Figure 24: Selenium analyses over time for Farm 7, Group 1

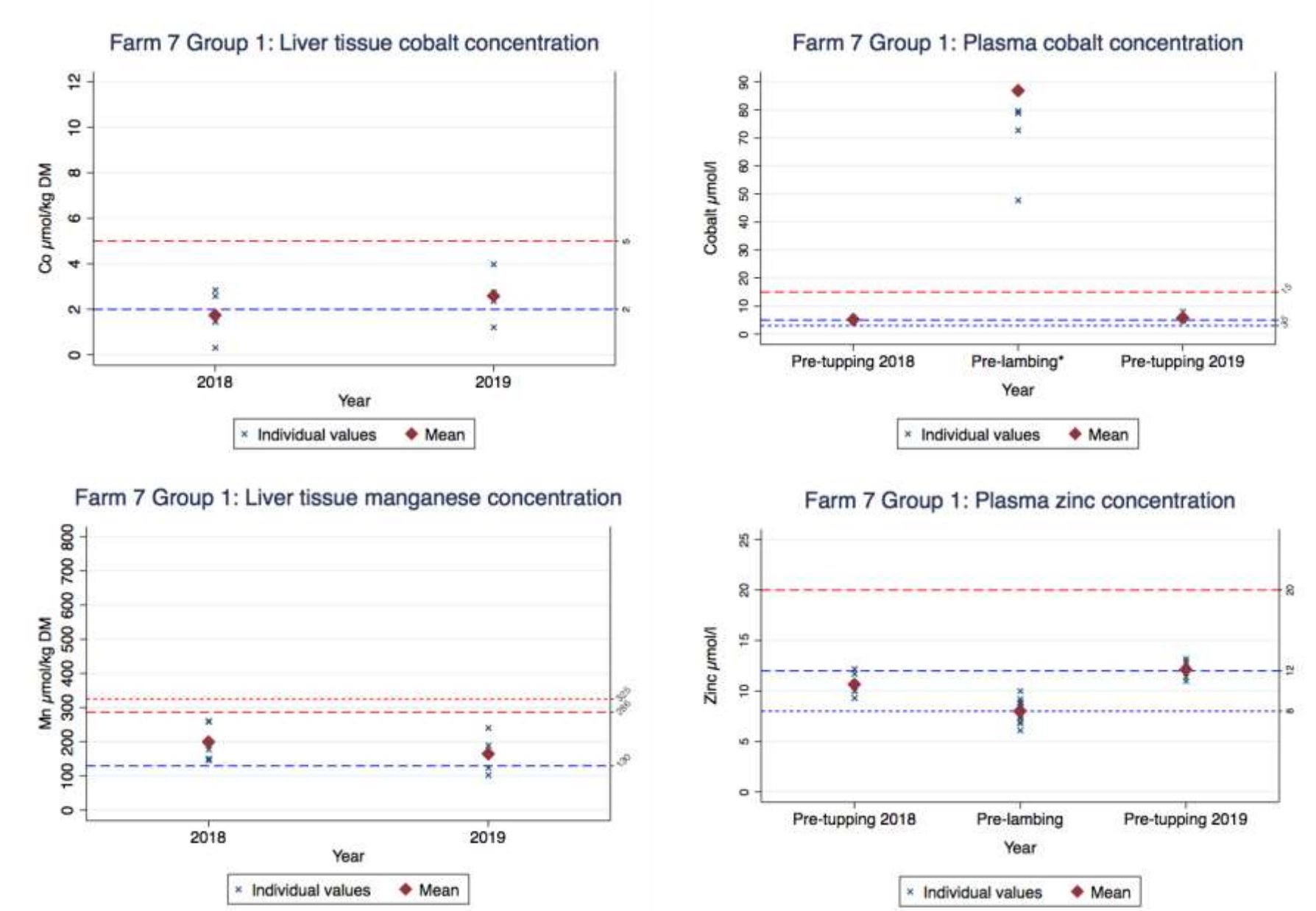
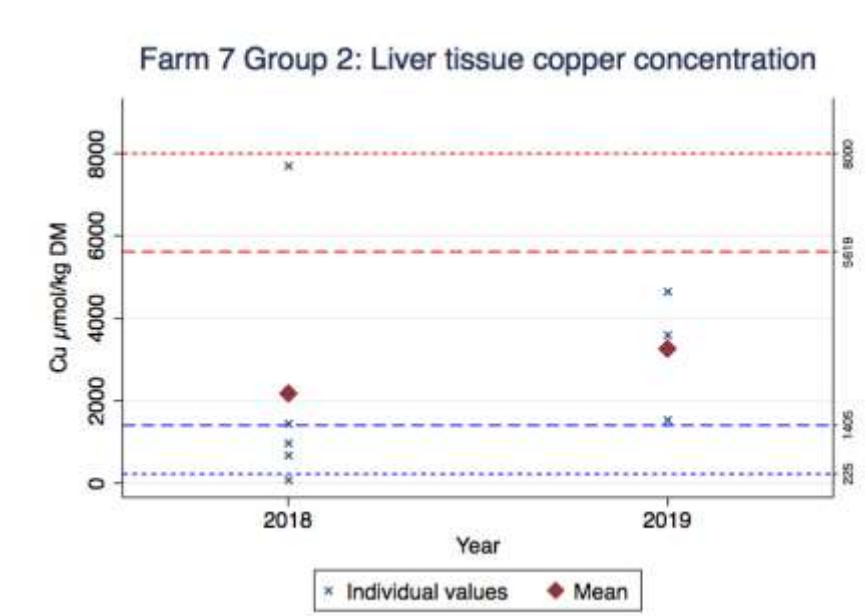
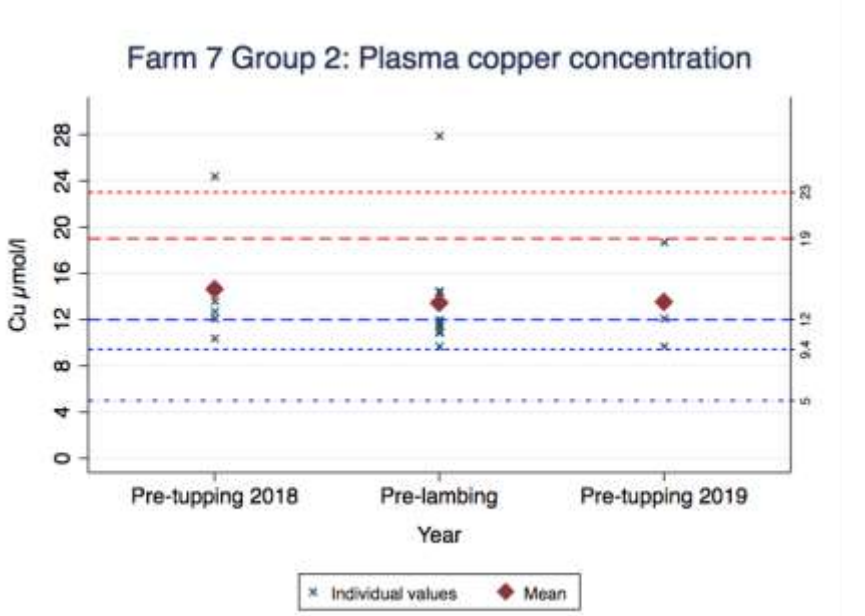
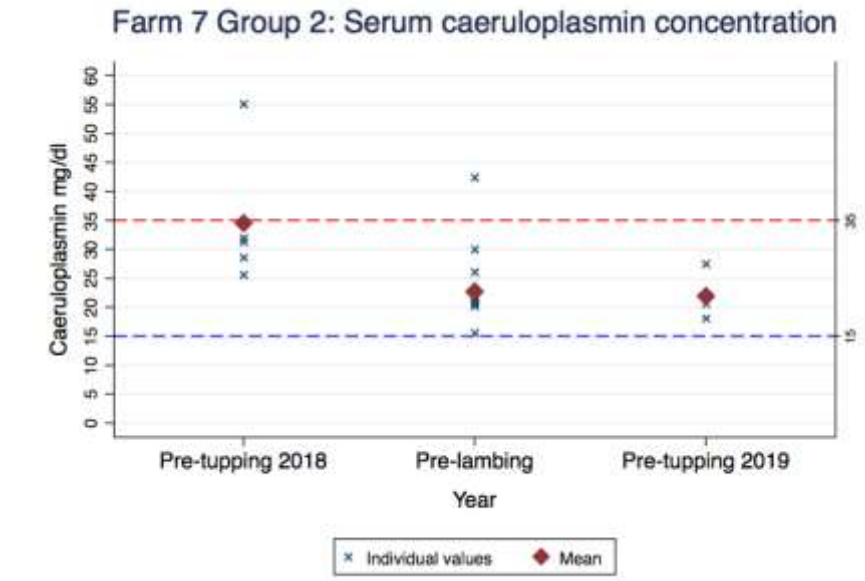


Figure 25: Cobalt, manganese and zinc analyses over time for Farm 7, Group 1; *in the interests of clarity, some very high values in the pre-lambing samples have been omitted.







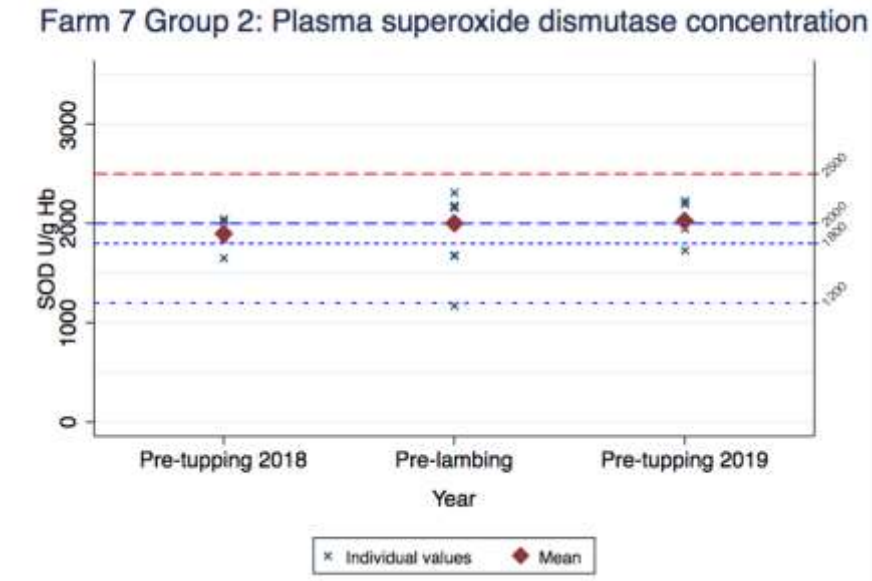


Figure 26: Copper analyses over time for Farm 7, Group 2

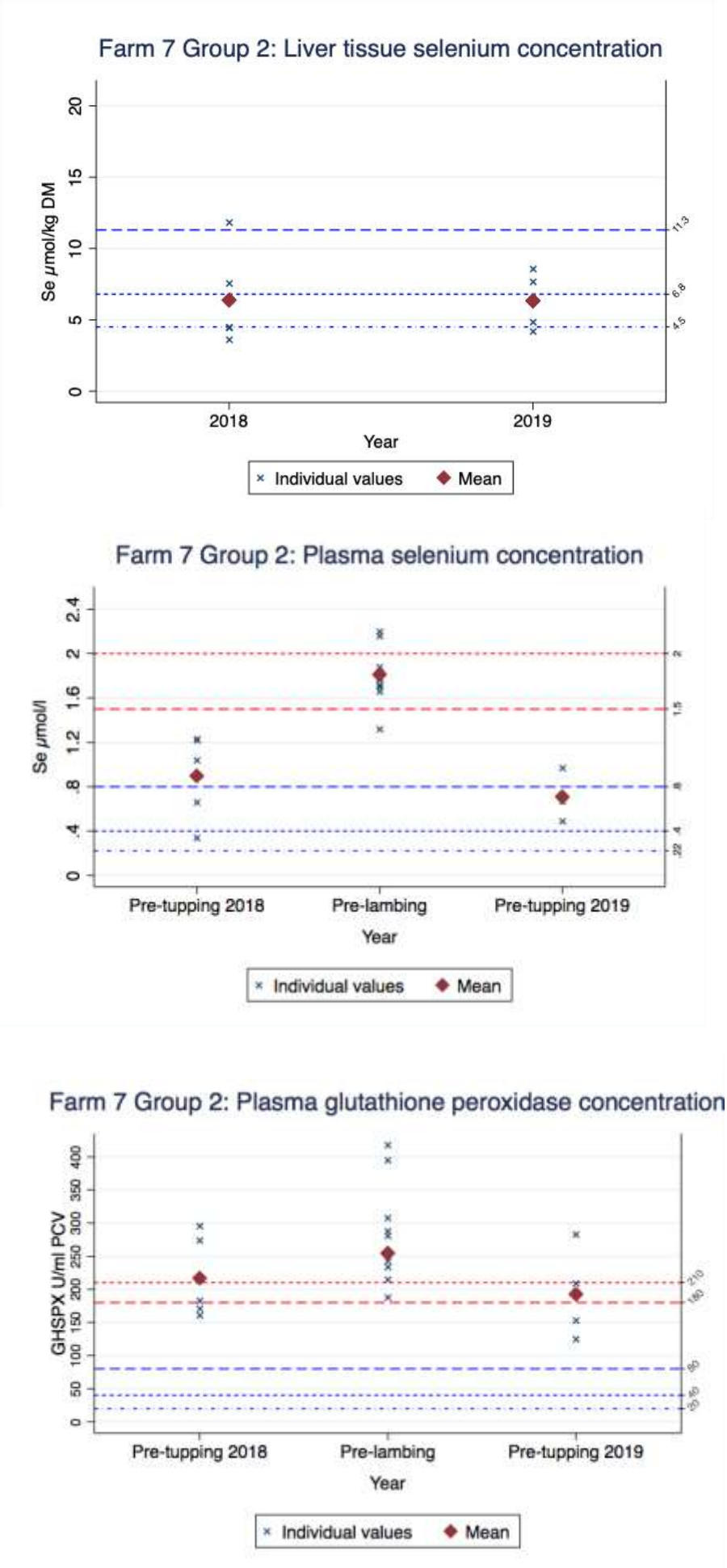
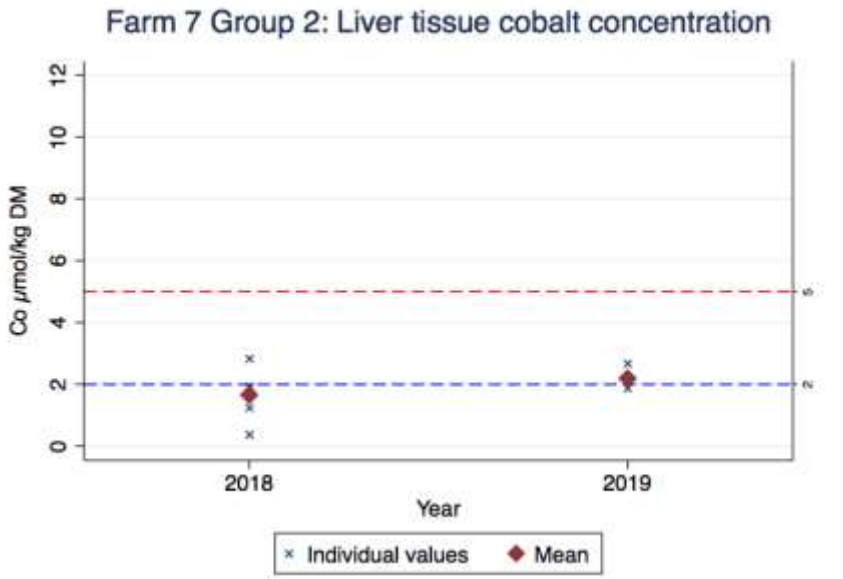
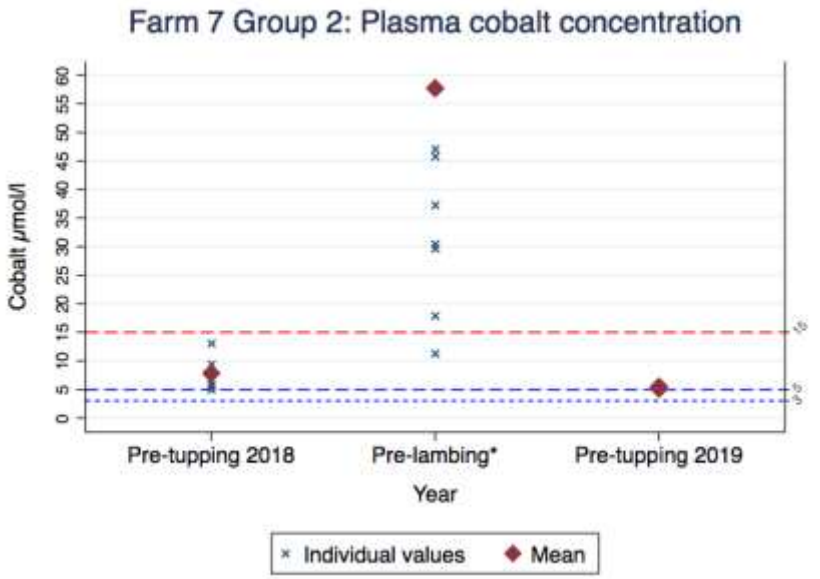
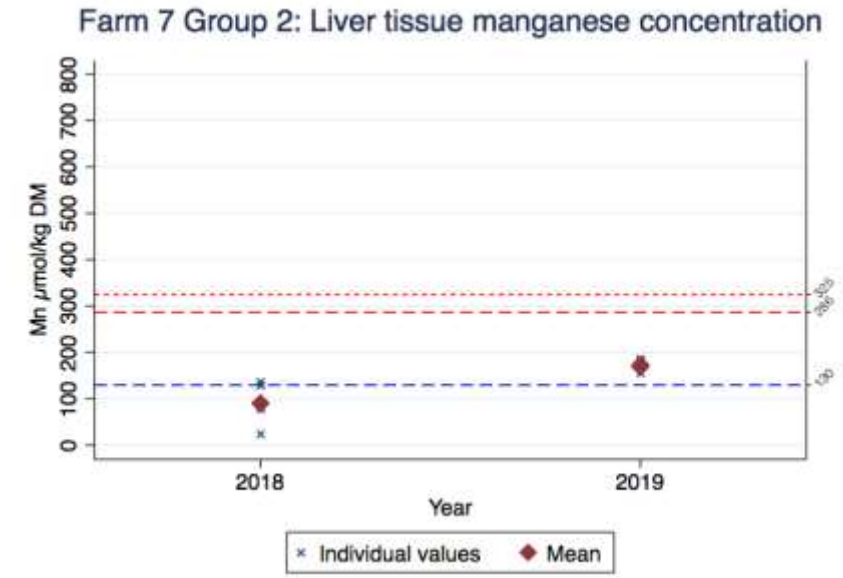


Figure 27: Selenium analyses over time for Farm 7, Group 2







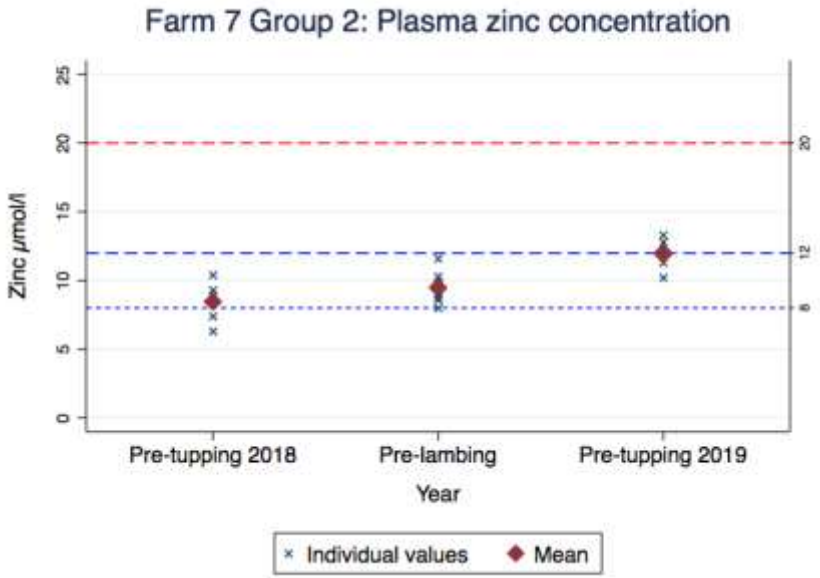


Figure 28: Cobalt, manganese and zinc analyses over time for Farm 7, Group 2; *in the interests of clarity, some very high values in the pre-lambing samples have been omitted.

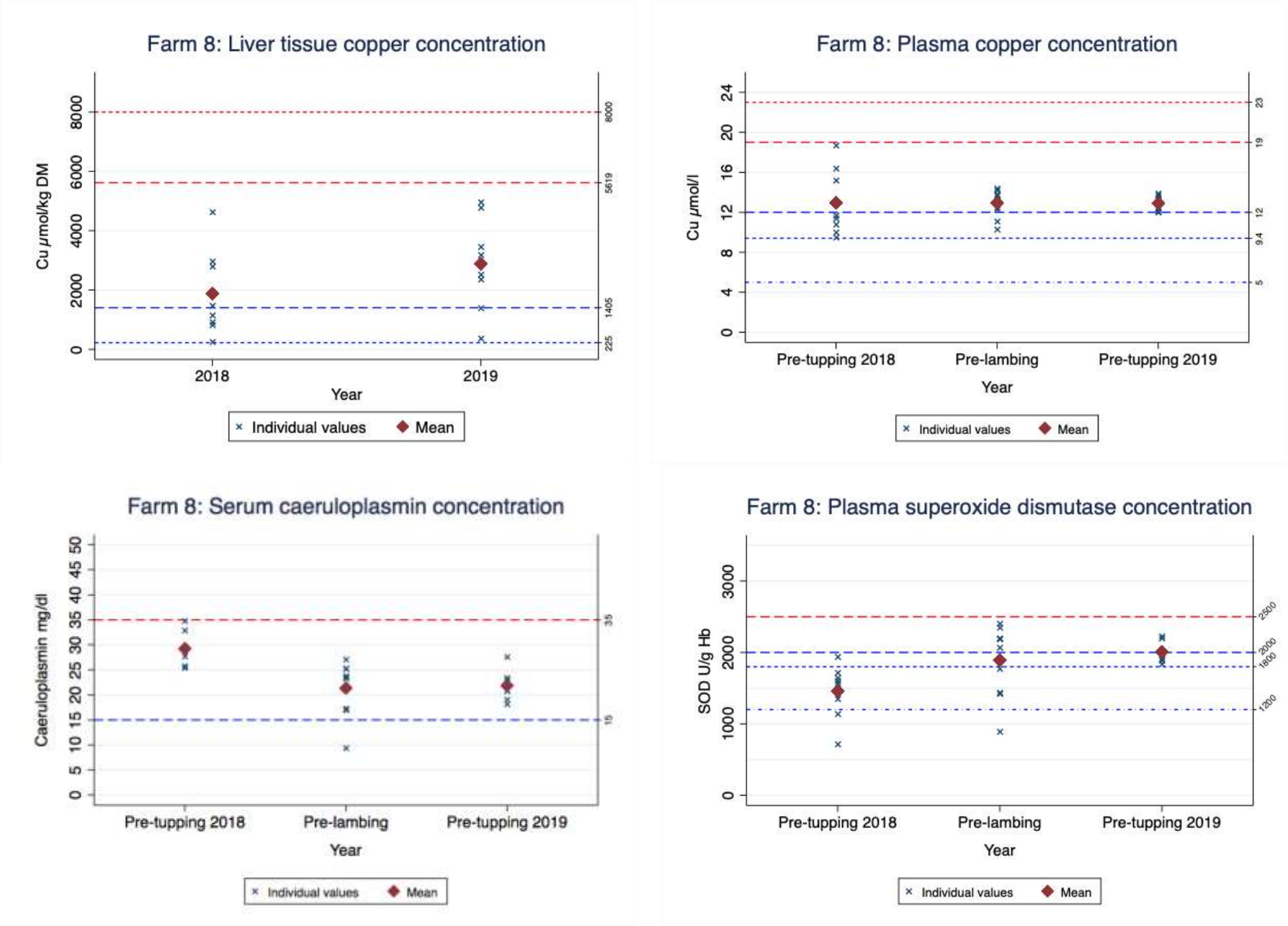
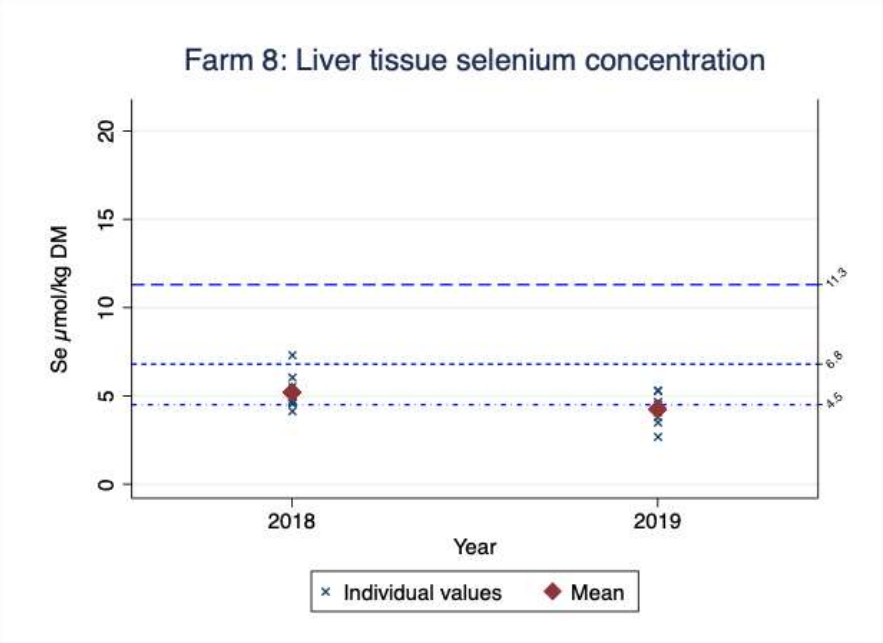
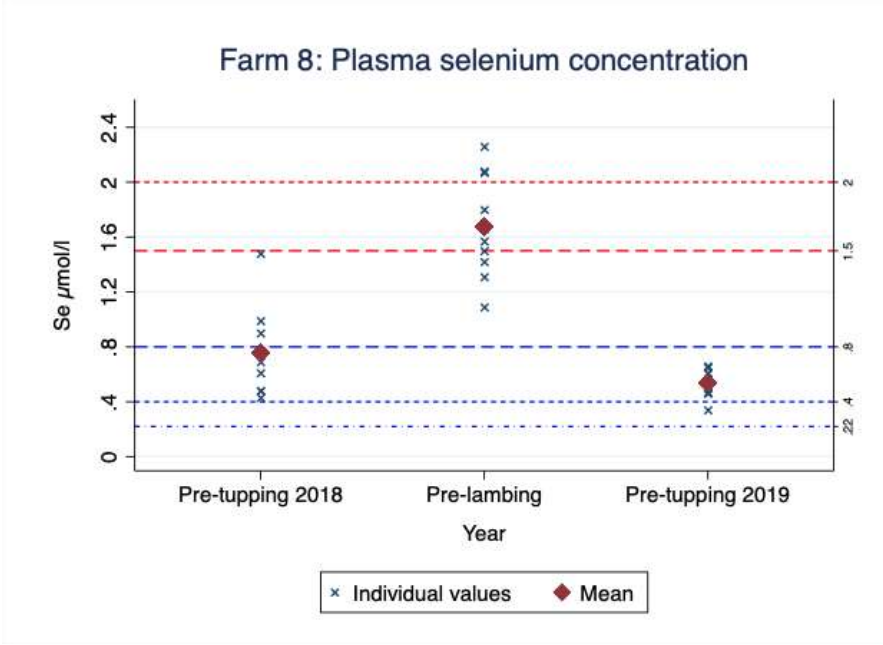


Figure 29: Copper analyses over time for Farm 8





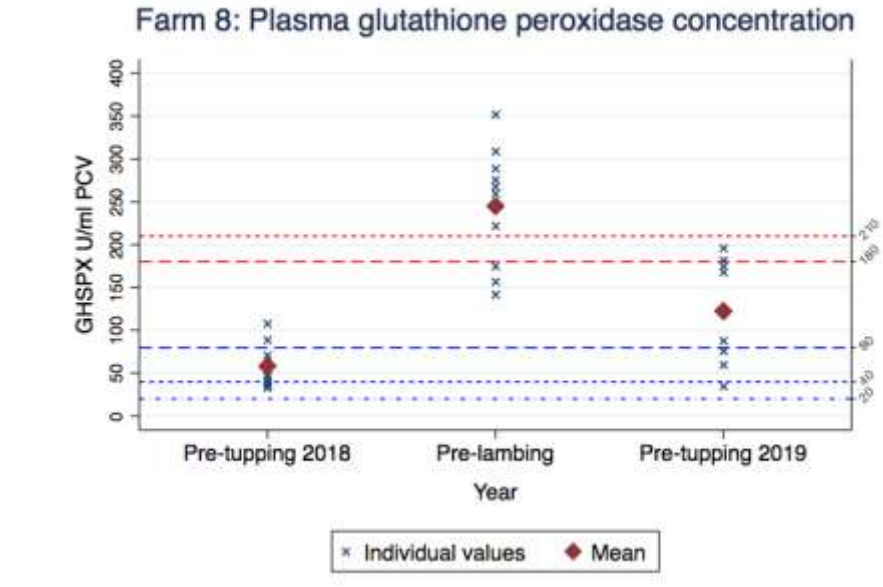
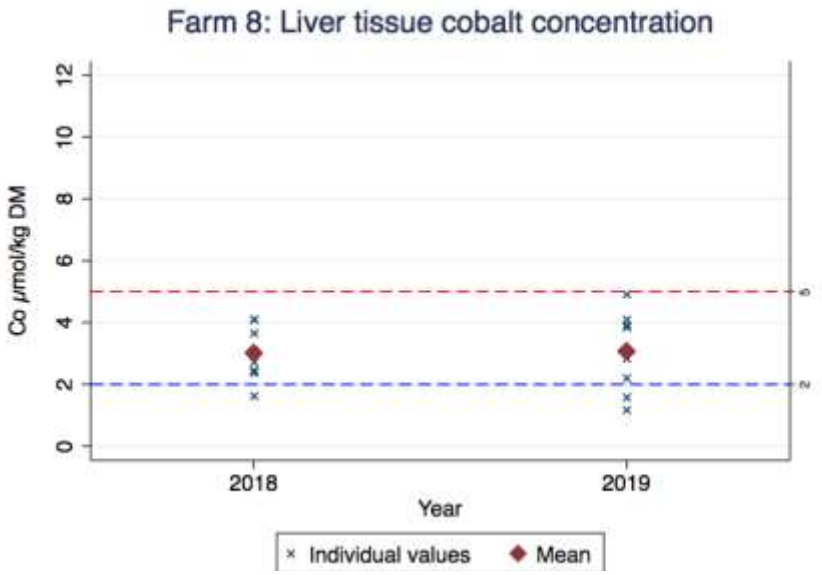
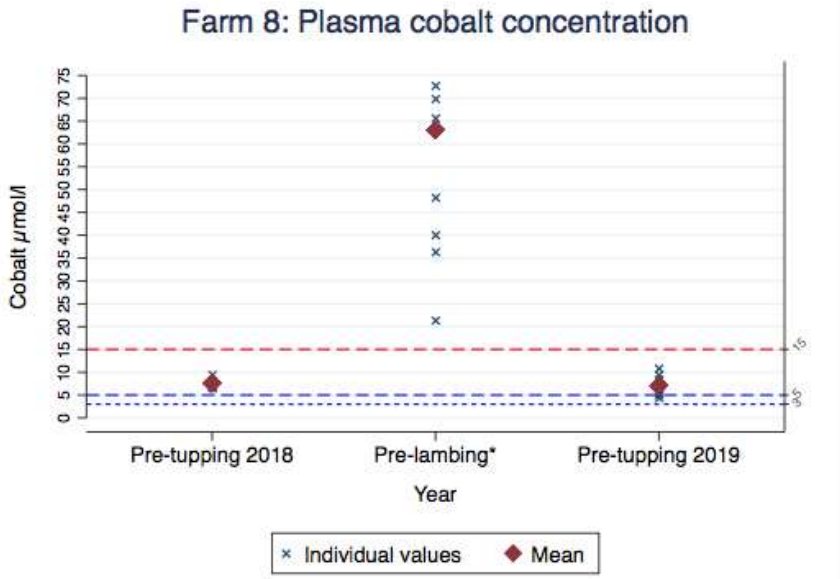
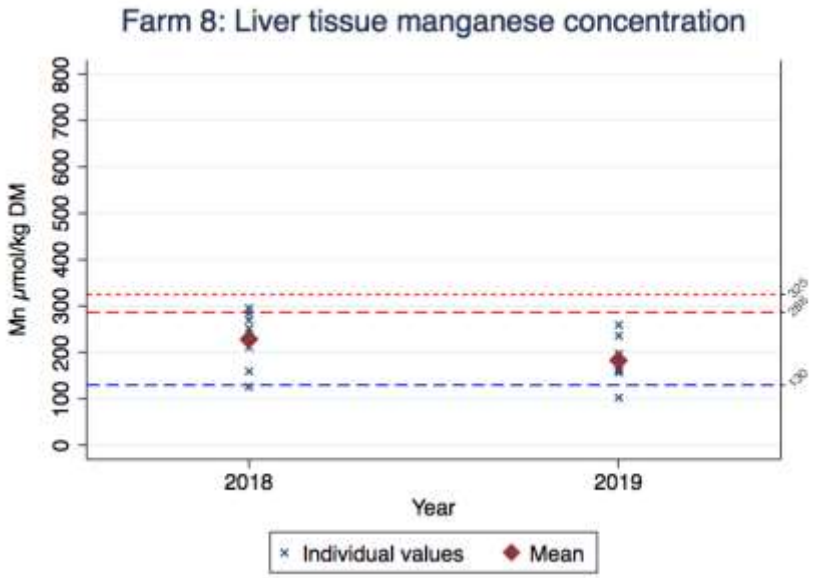


Figure 30: Selenium analyses over time for Farm 8







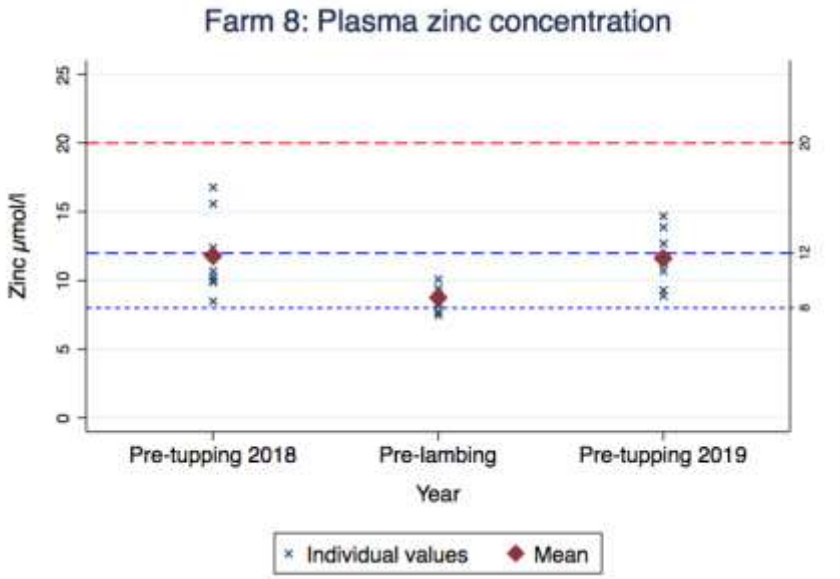
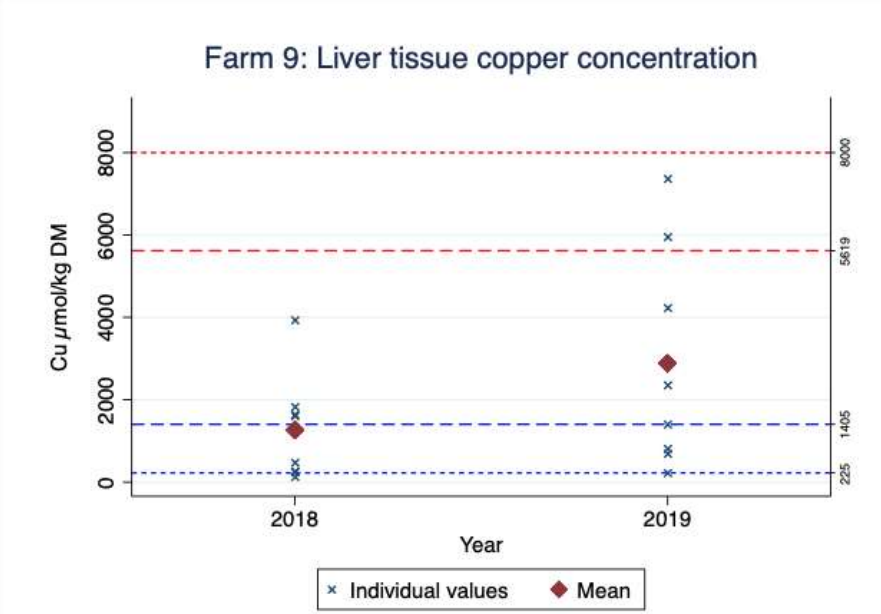
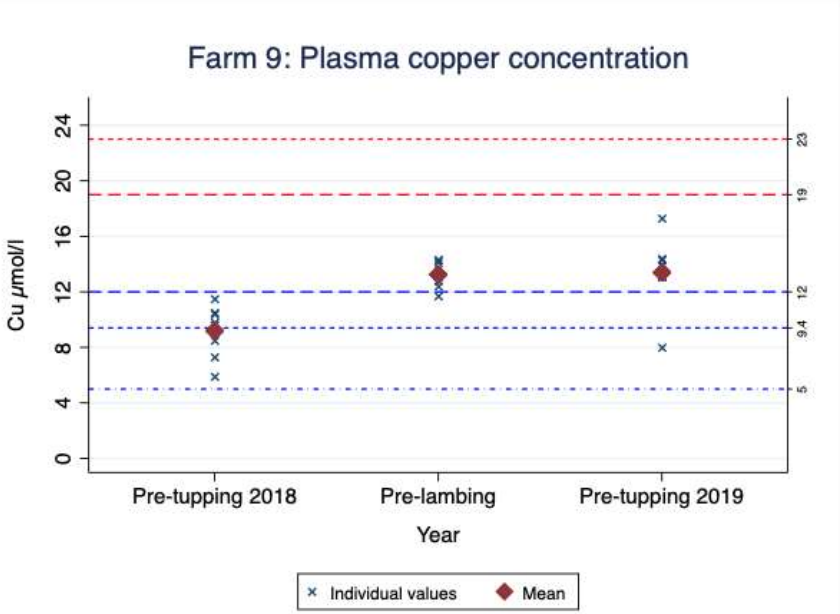
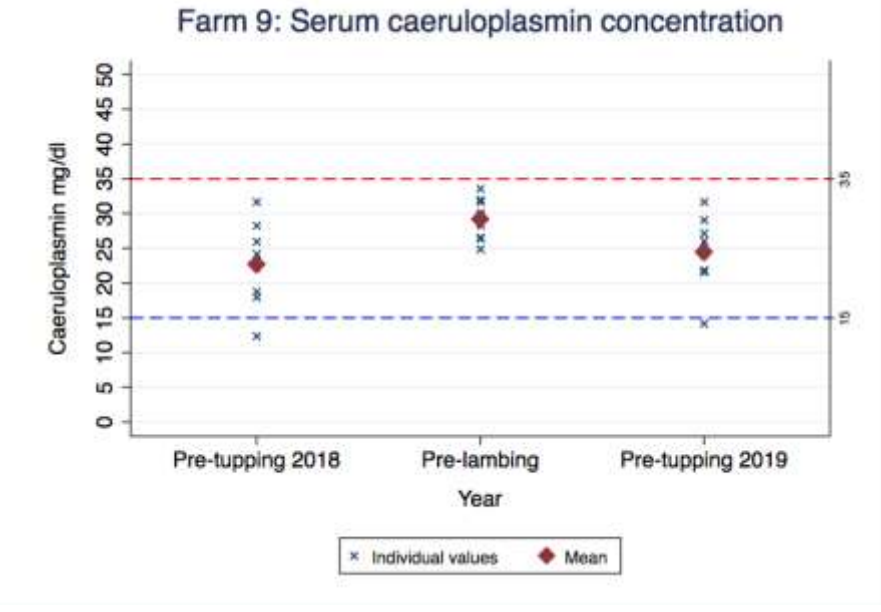


Figure 31: Cobalt, manganese and zinc analyses over time for Farm 8; *in the interests of clarity, some very high values in the pre-lambing samples have been omitted.







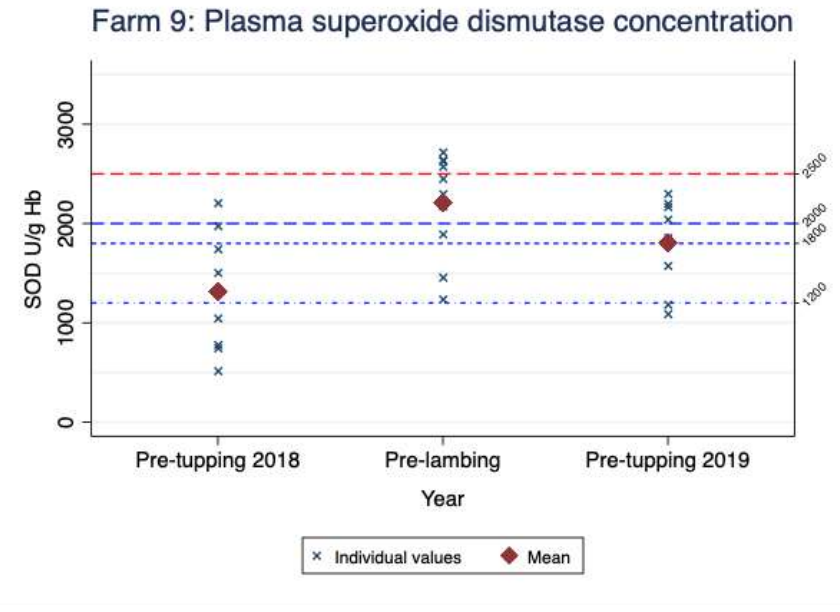


Figure 32: Copper analyses over time for Farm 9

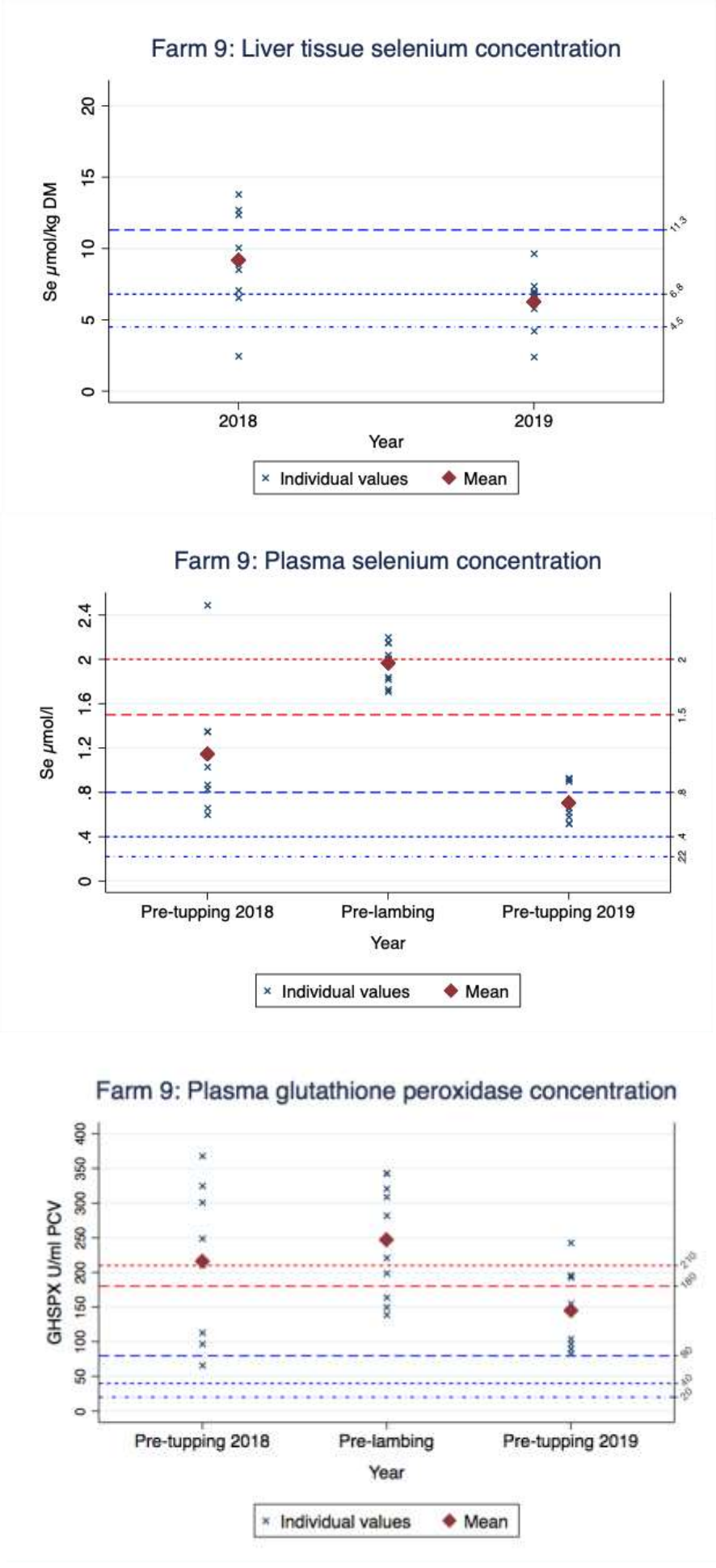


Figure 33: Selenium analyses over time for Farm 9

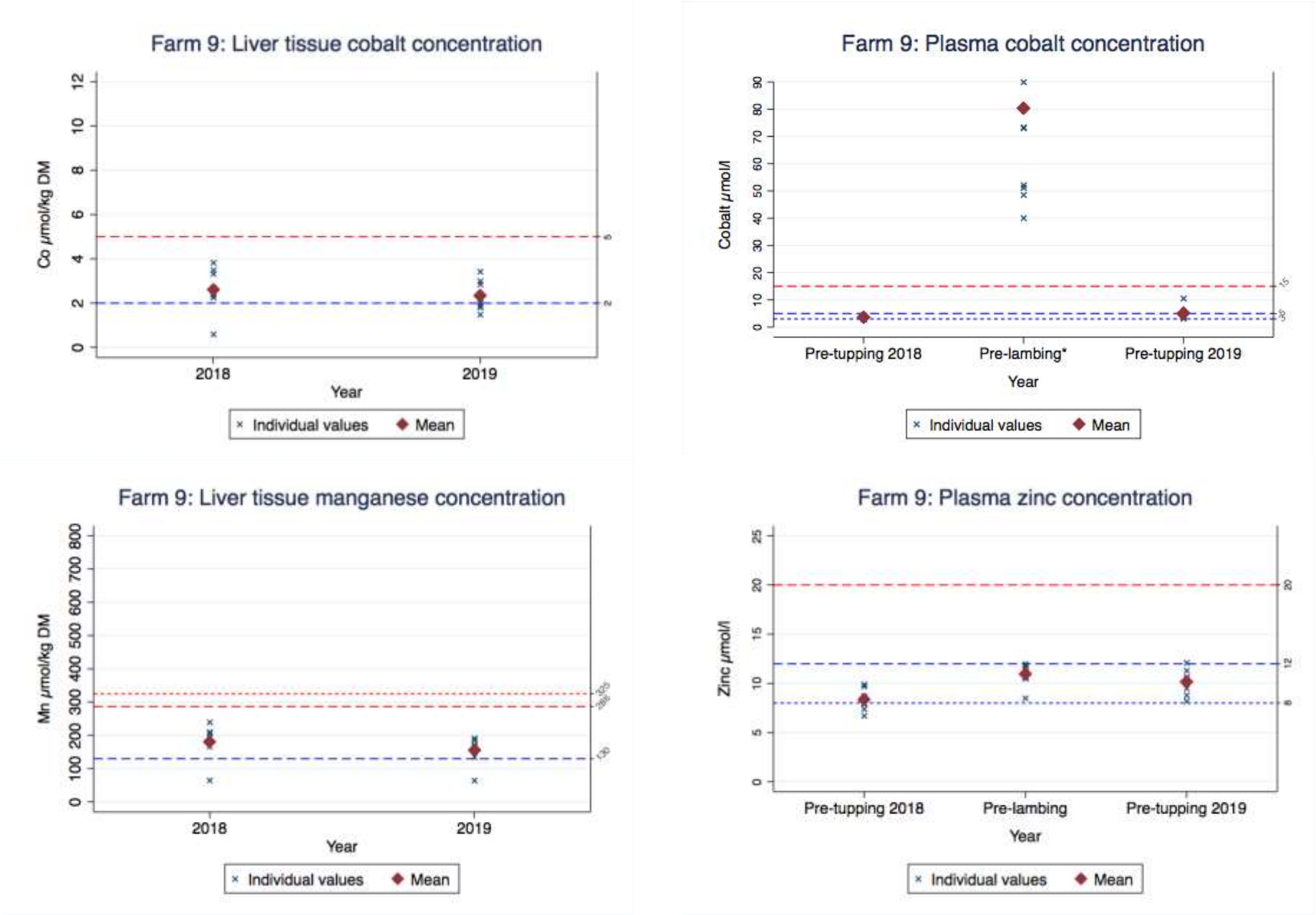
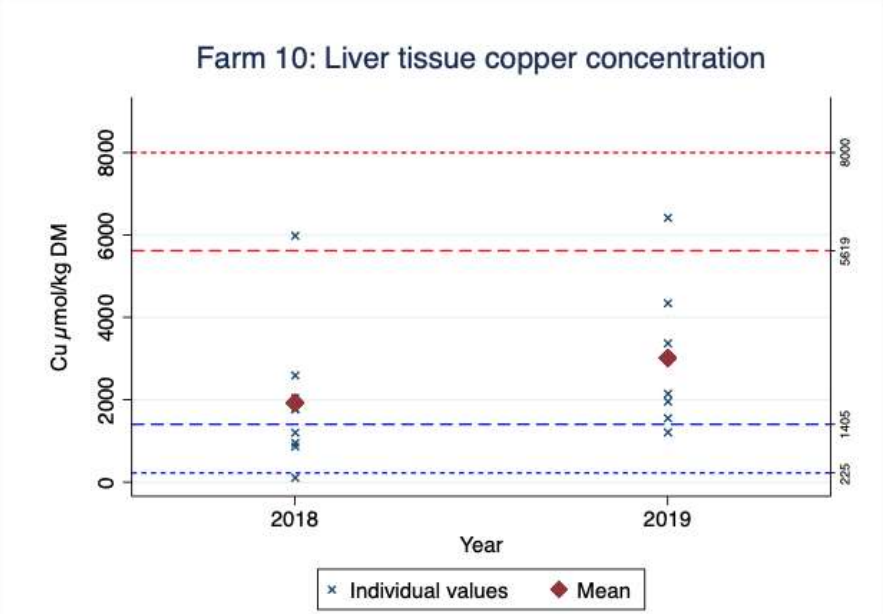
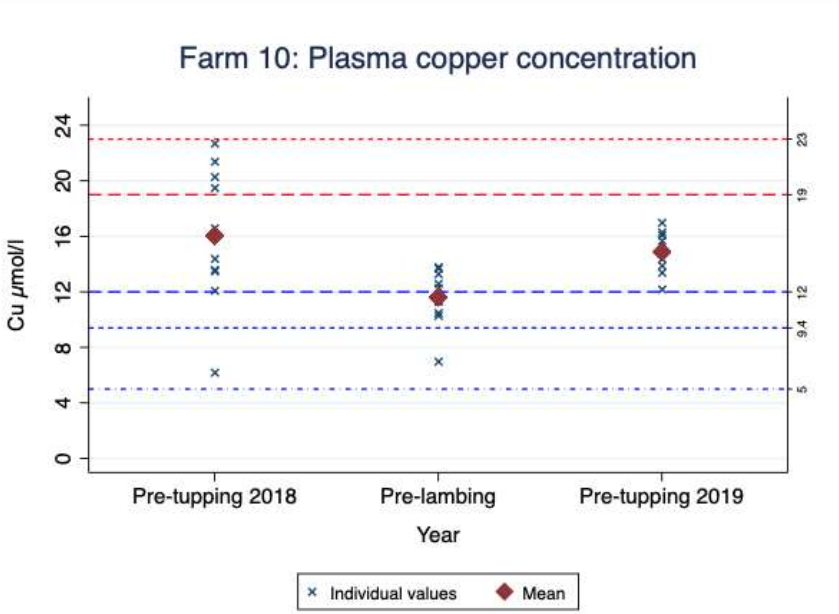
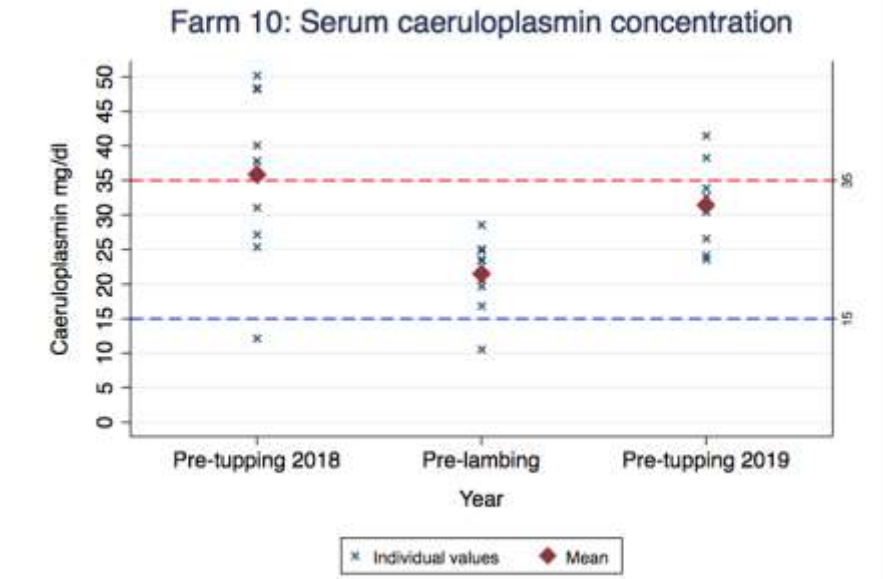


Figure 34: Cobalt, manganese and zinc analyses over time for Farm 9; *in the interests of clarity, some very high values in the pre-lambing samples have been omitted.







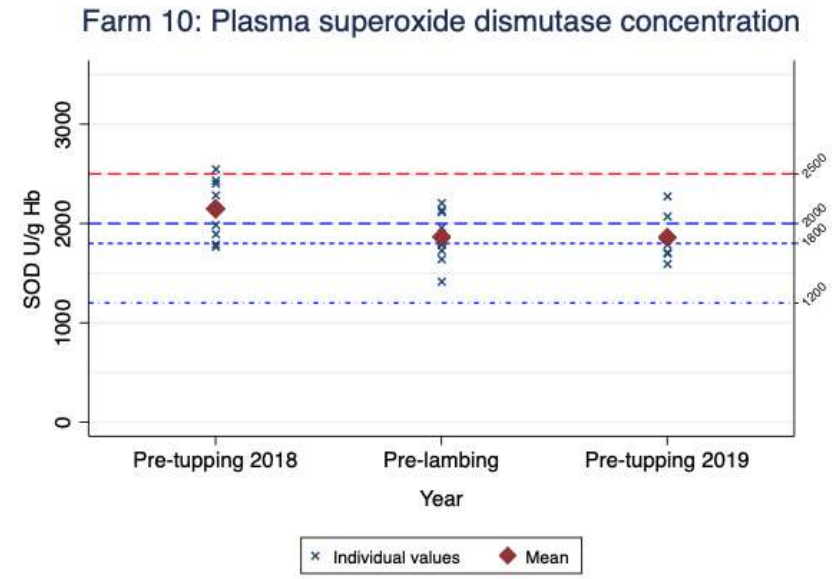
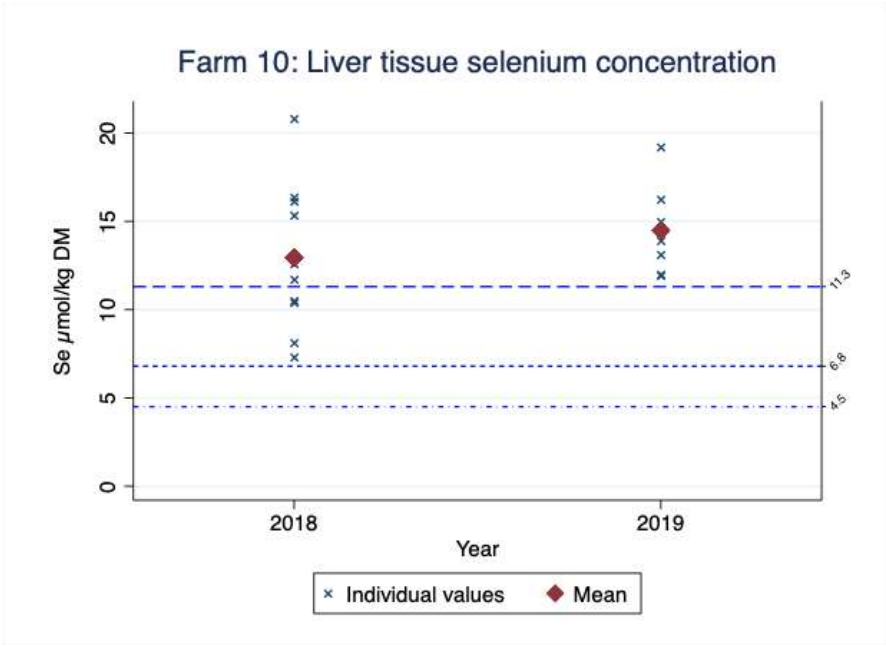
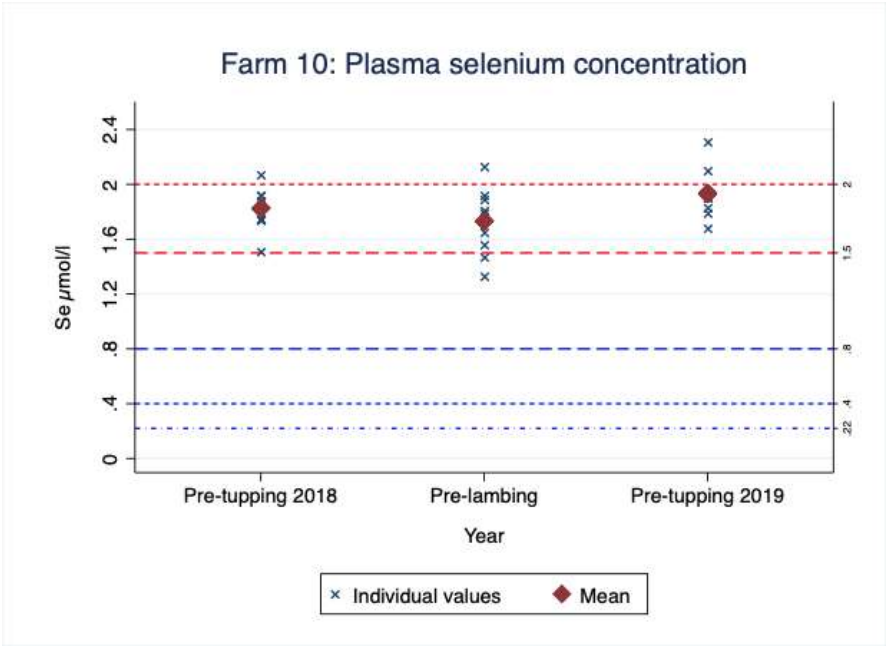


Figure 35: Copper analyses over time for Farm 10





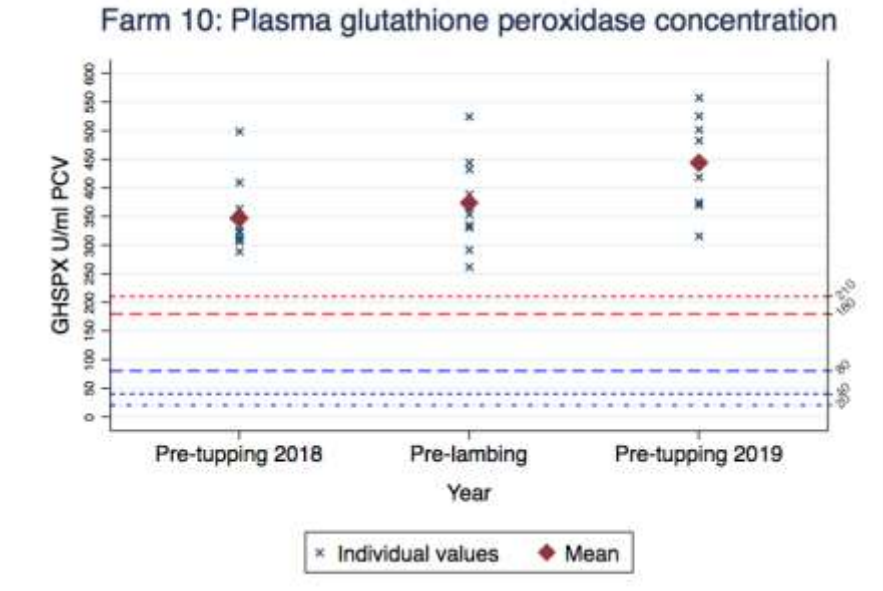
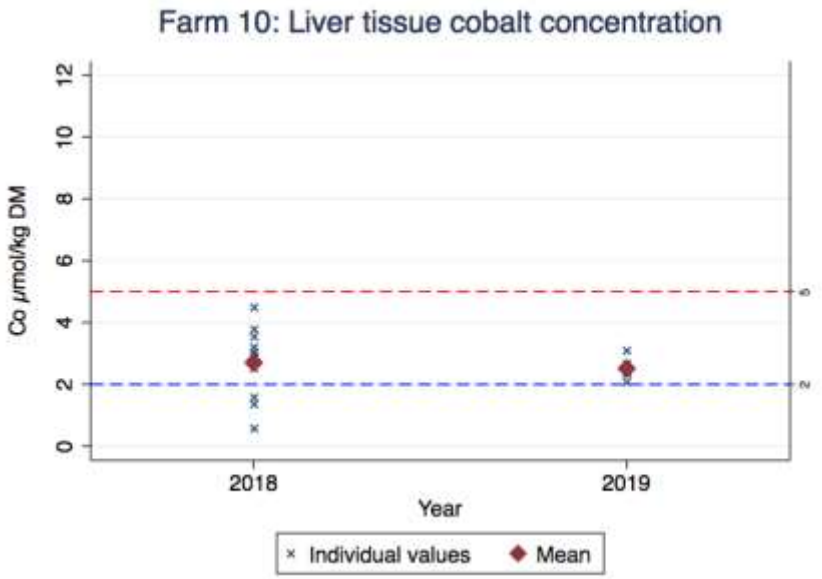
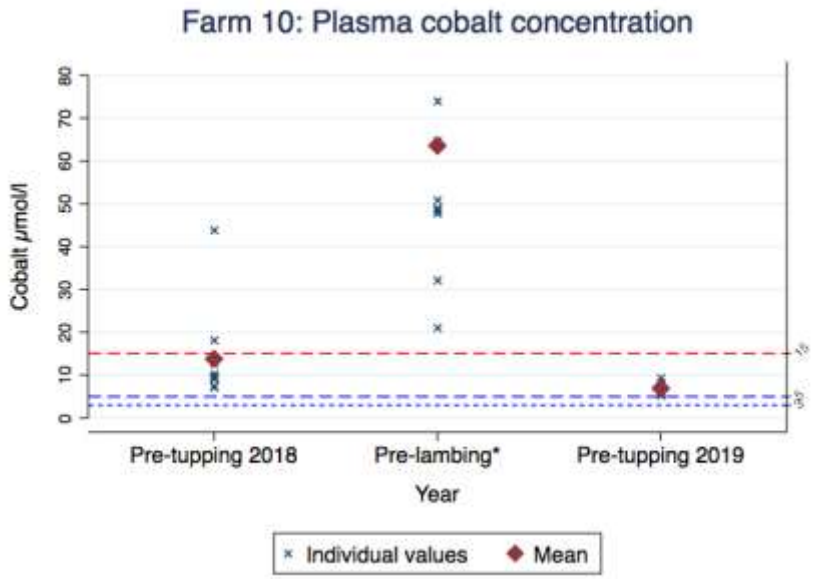
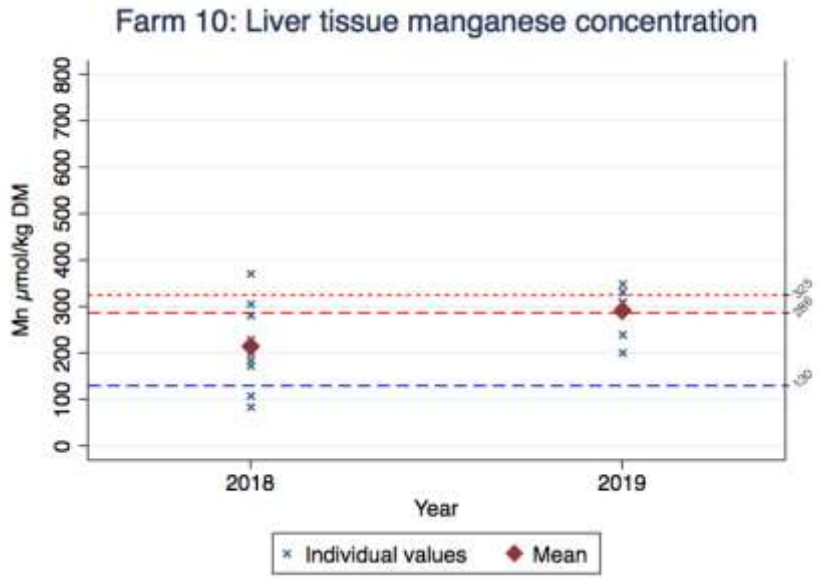


Figure 36: Selenium analyses over time for Farm 10







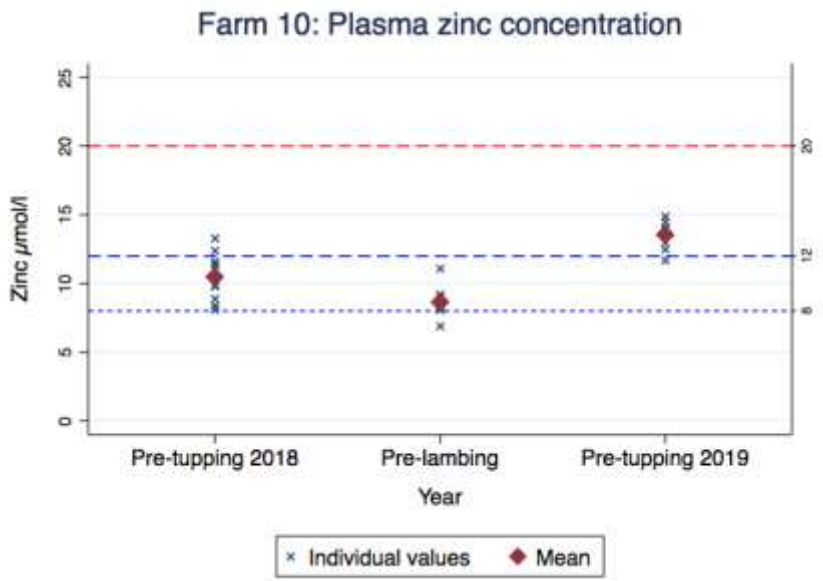
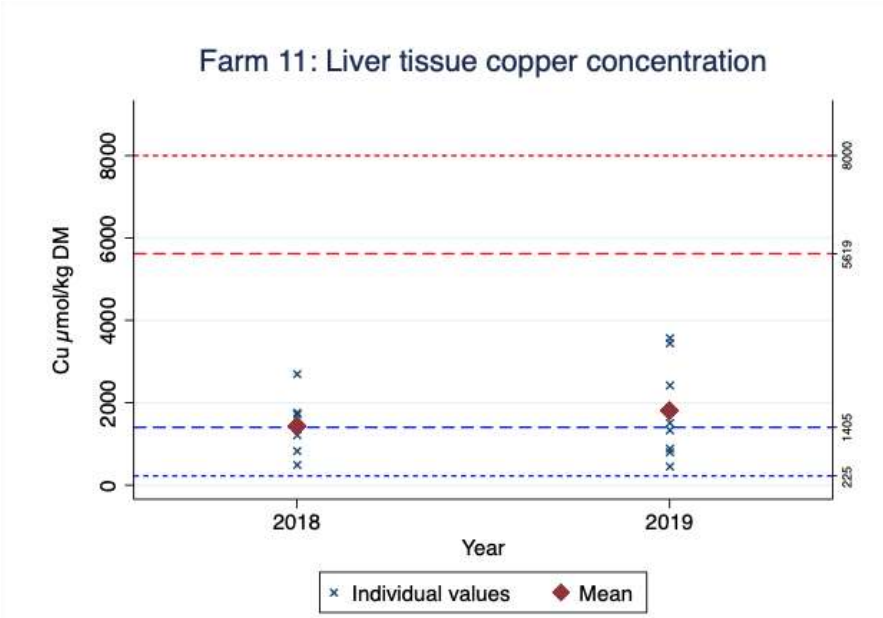
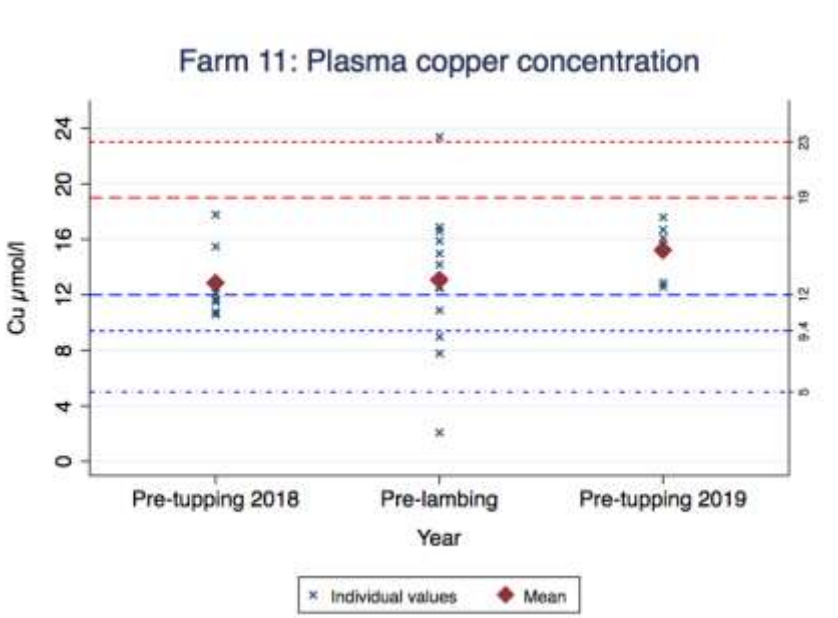
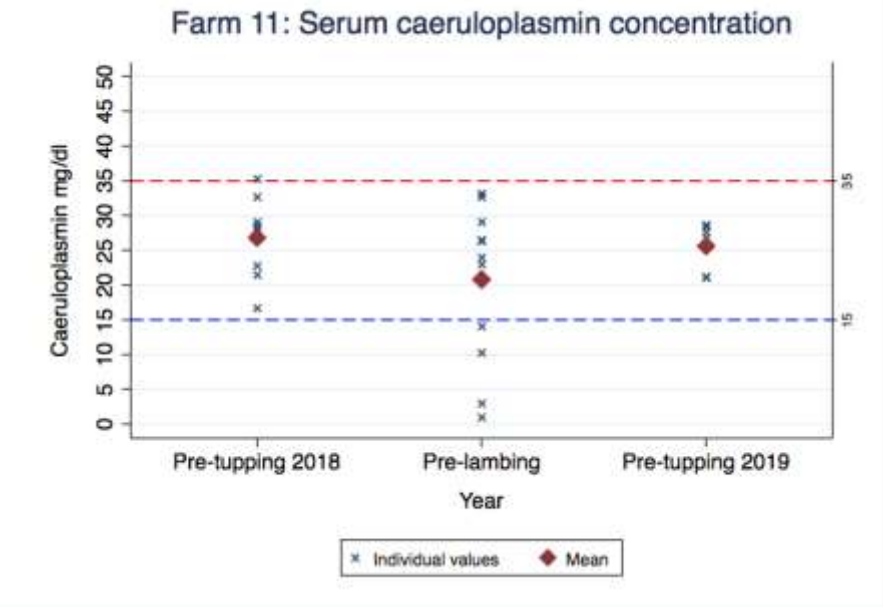


Figure 37: Cobalt, manganese and zinc analyses over time for Farm 10; *in the interests of clarity, some very high values in the pre-lambing samples have been omitted.







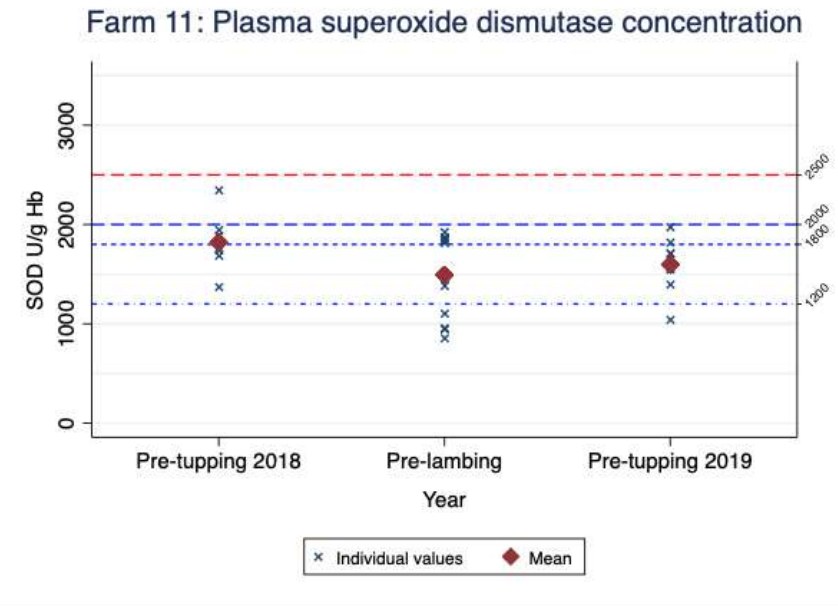
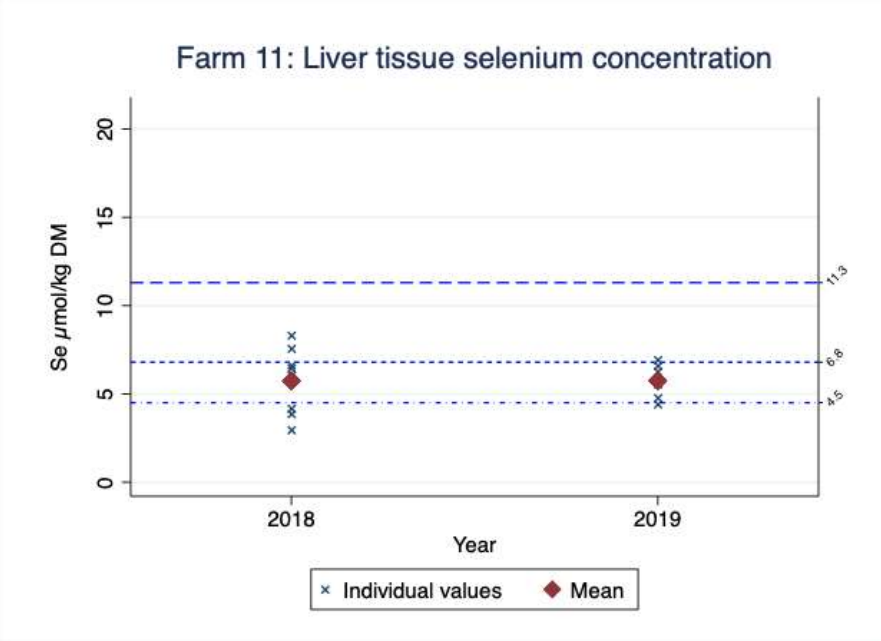
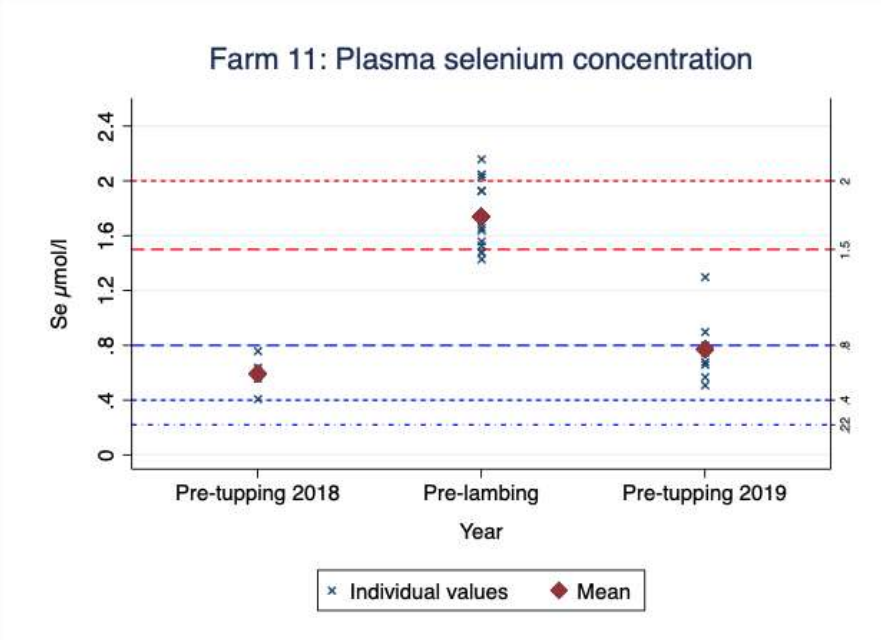


Figure 38: Copper analyses over time for Farm 11





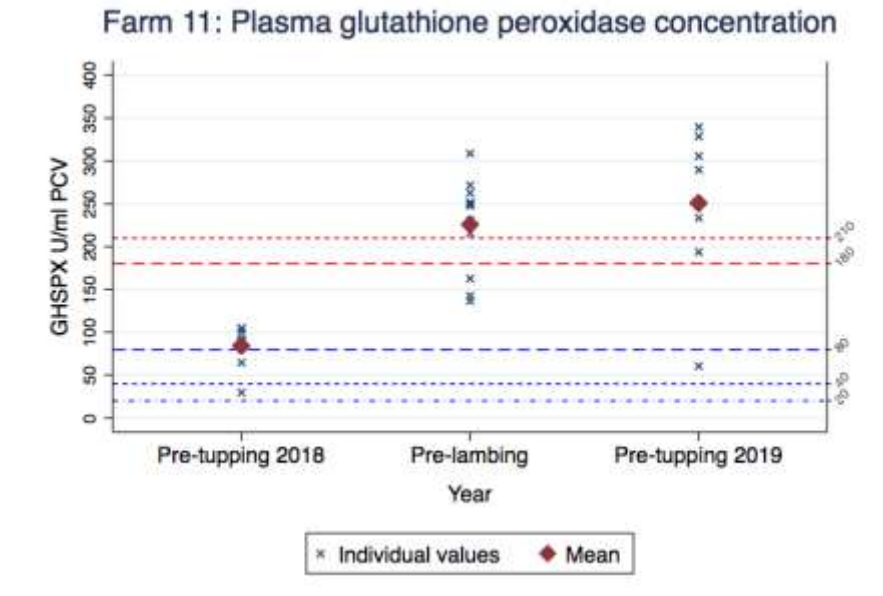
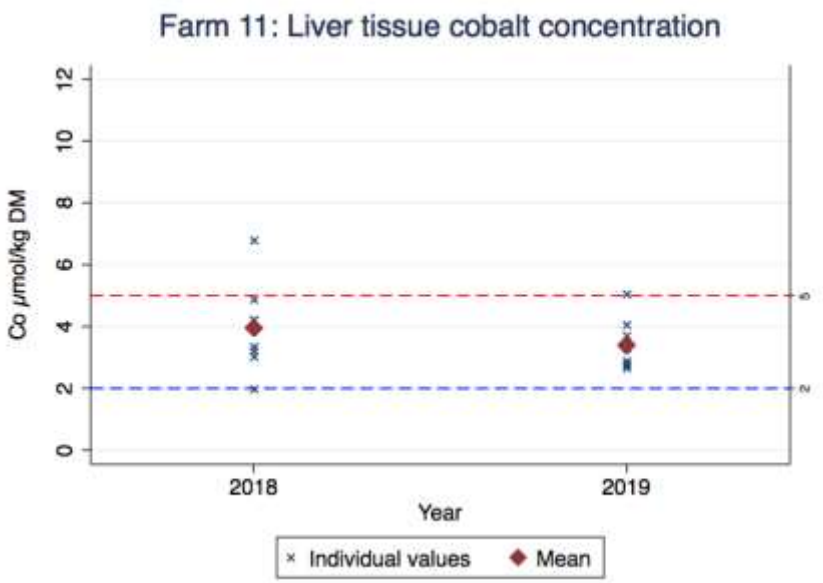
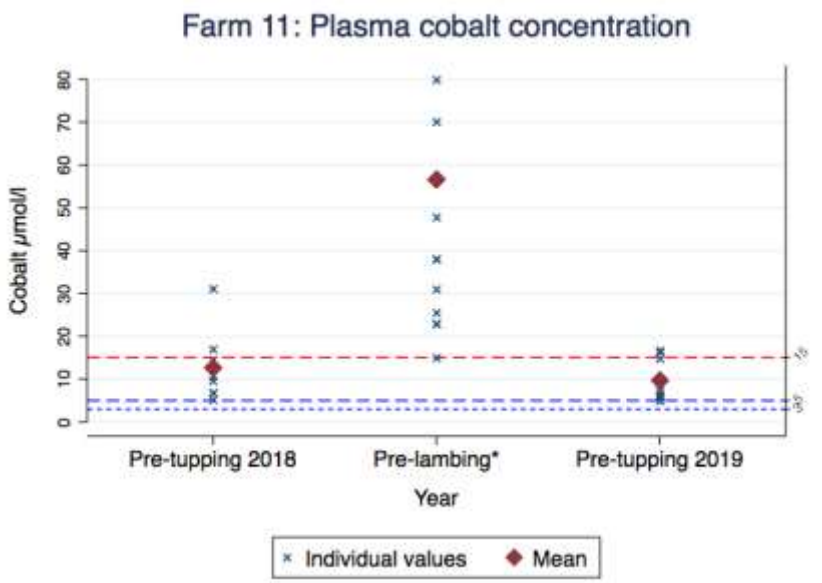
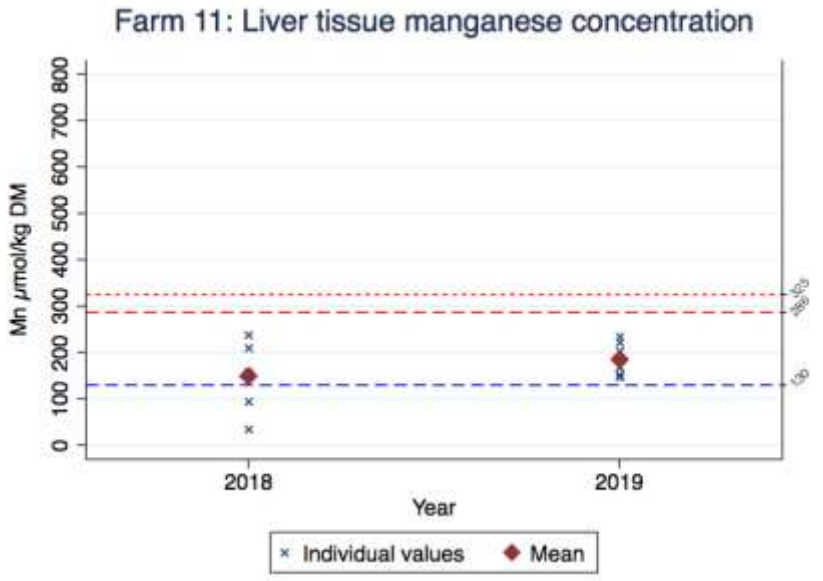


Figure 39: Selenium analyses over time for Farm 11







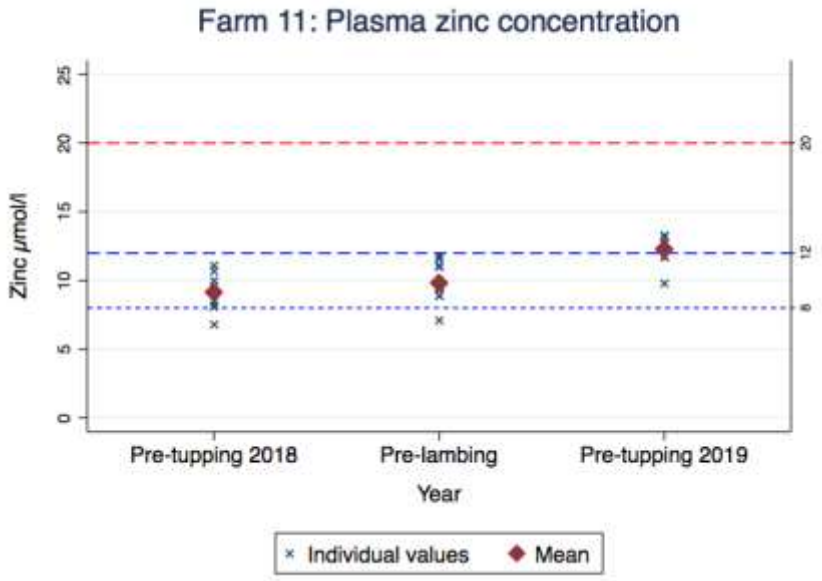
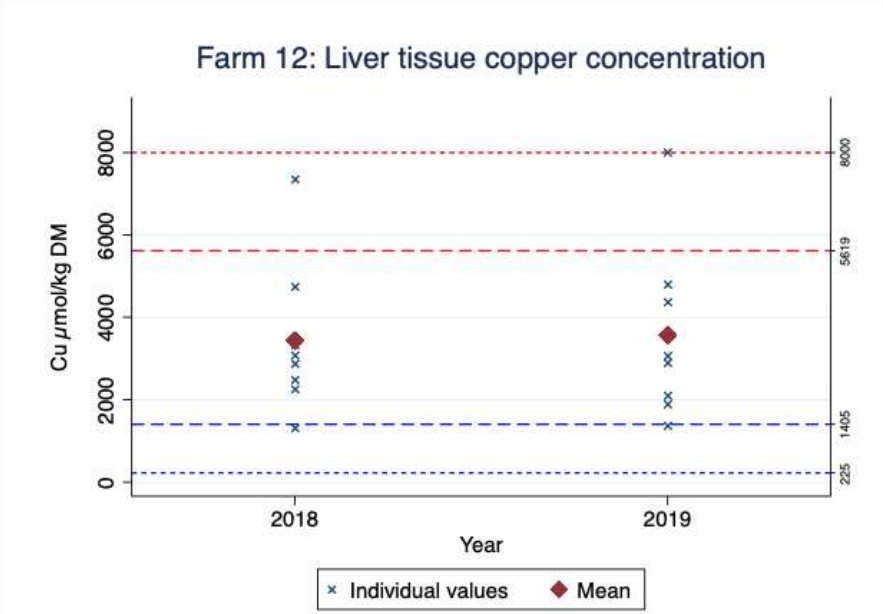
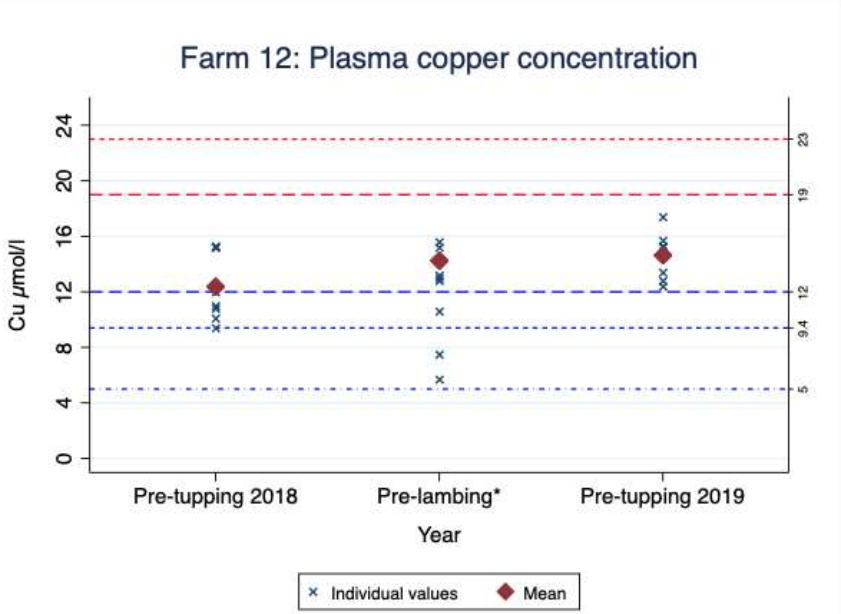
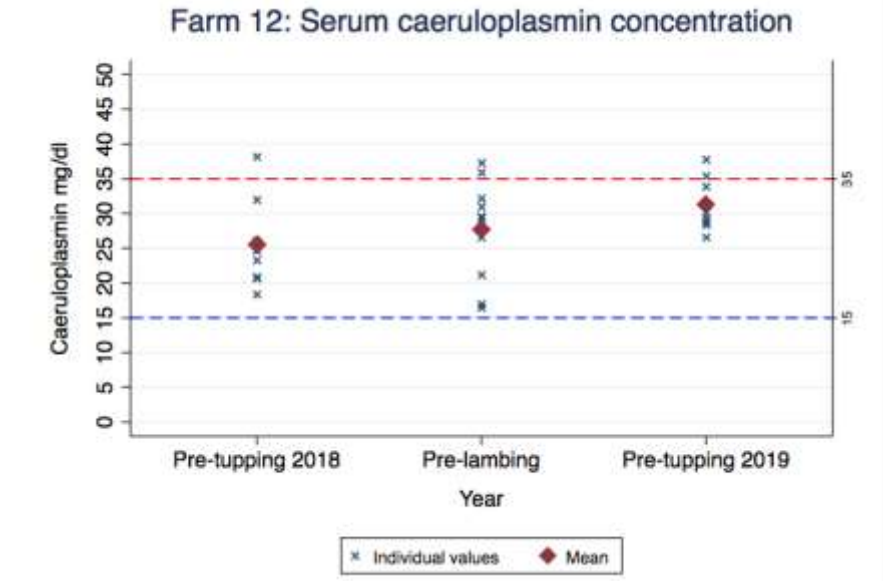


Figure 40: Cobalt, manganese and zinc sulphate over time for Farm 11; *in the interests of clarity, some very high values in the pre-lambing samples have been omitted.







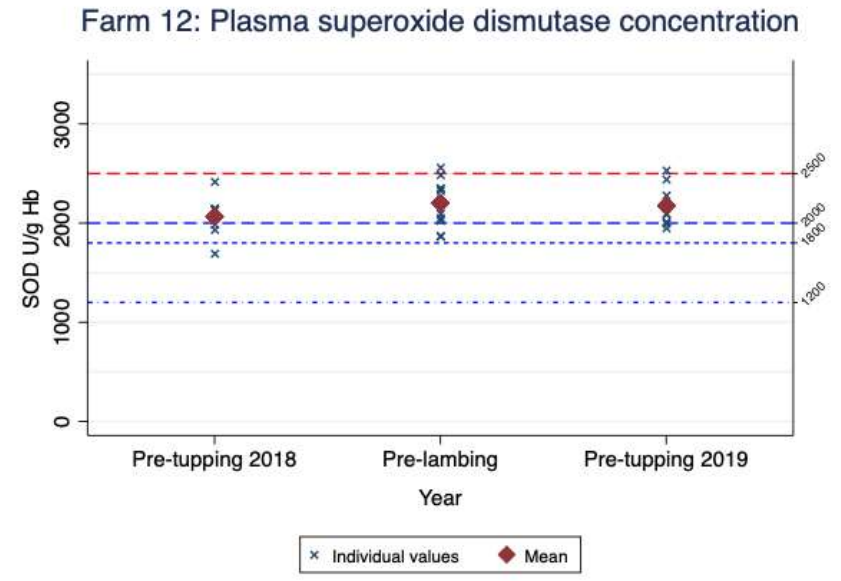


Figure 41: Copper analyses over time for Farm 12; *in the interests of clarity, some very high values in the pre-lambing samples have been omitted.

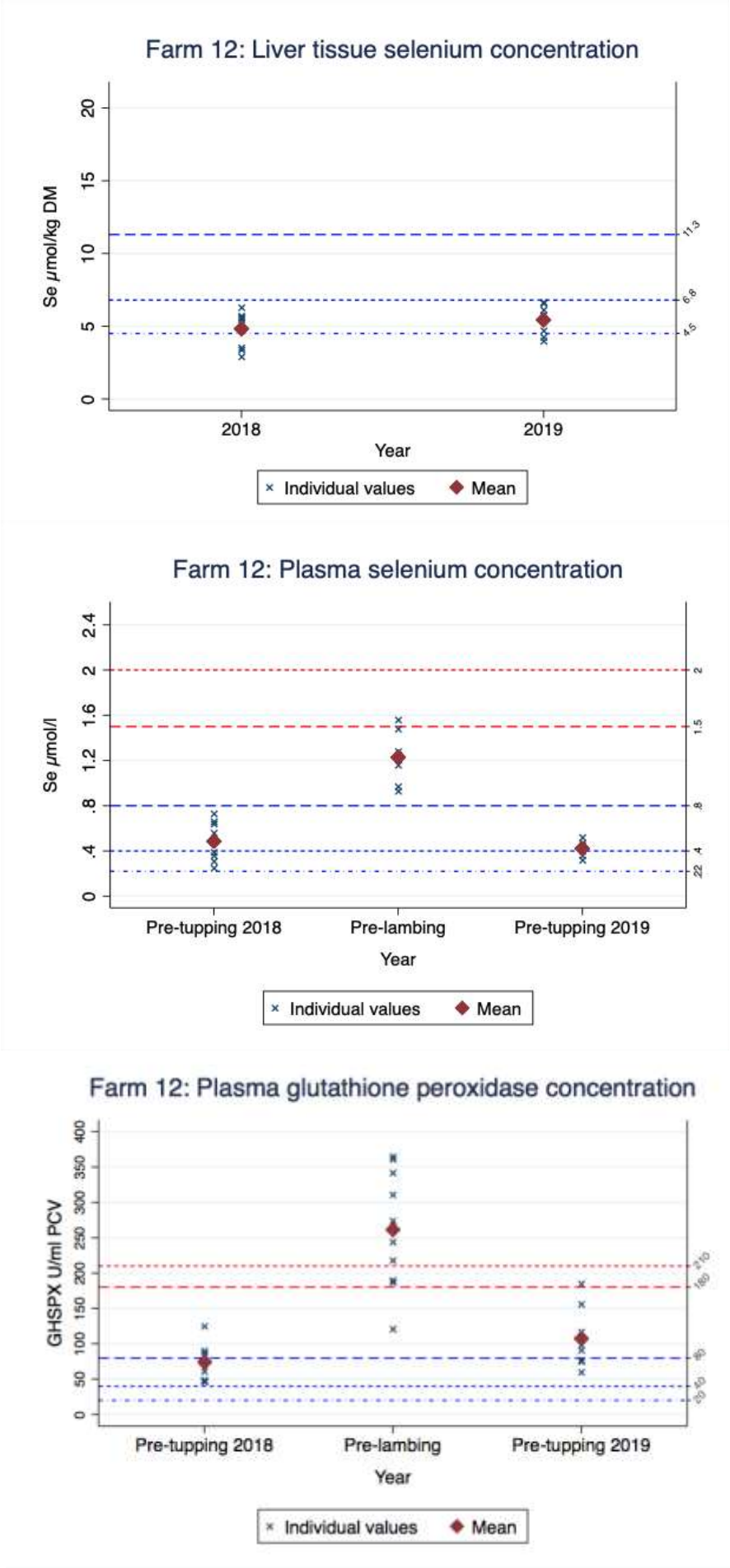


Figure 42: Selenium analyses over time for Farm 12

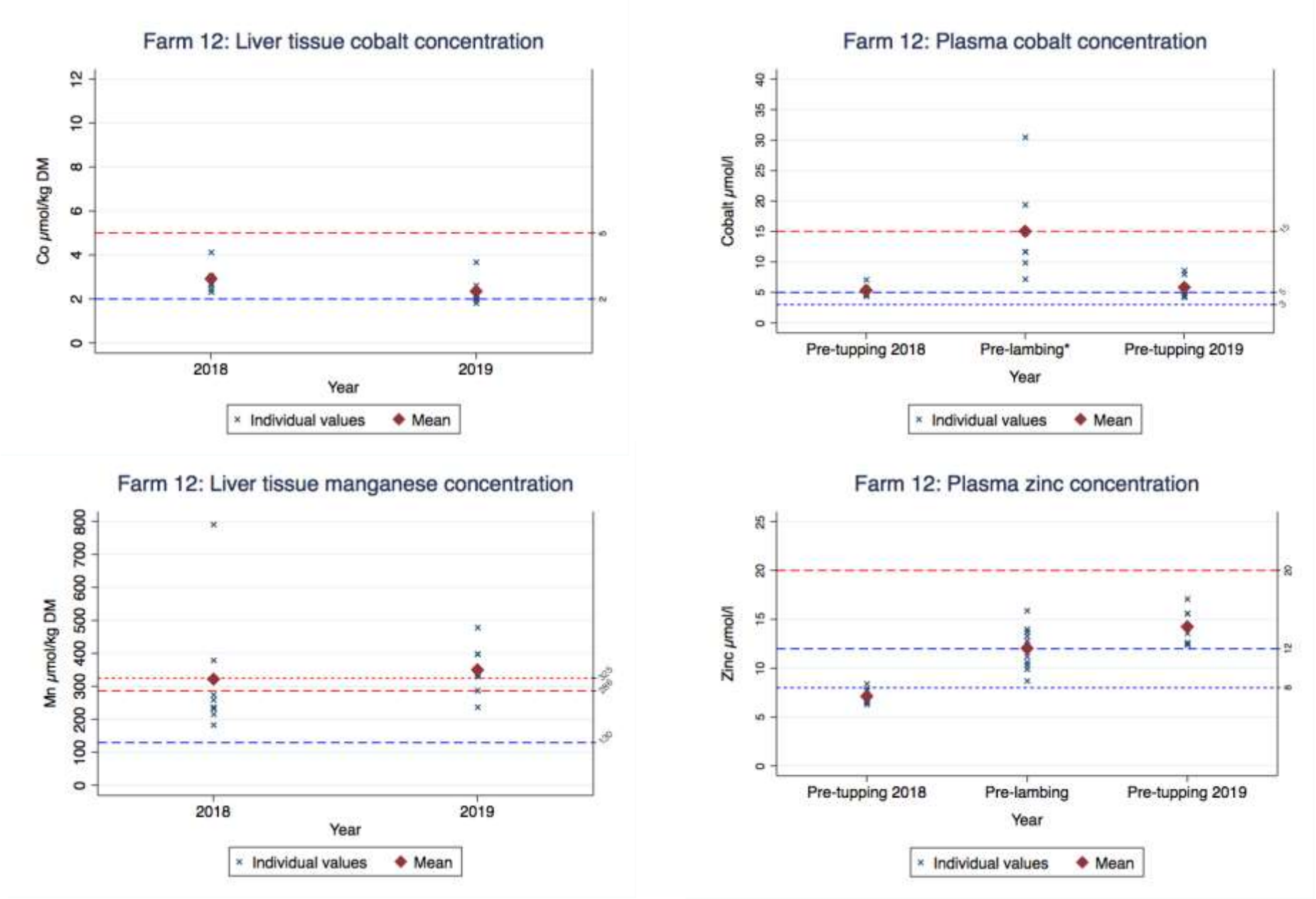


Figure 43: Cobalt, manganese and zinc analyses over time for Farm 12; *in the interests of clarity, some very high values in the pre-lambing samples have been omitted.

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