Working with farmers to optimise mineral balance in grazing sheep.

Background: Trace mineral analysis is a useful but under-utilised tool in ruminant livestock production. Routine on-farm use of diagnostic techniques can help ensure animal requirements are being met through improved supplementation choices and timing of diagnostics.

Aim of the article: This article discusses the practical importance of trace mineral testing and supplementation in sheep flocks with a case study of trace mineral management.

Key learning outcomes:

After reading this article you should have an understanding of;

- ☐ The importance of collecting routine blood, liver and forage samples in the development of a supplemental strategy.
- ☐ The role different blood and liver analytes play in mineral status determination.
- ☐ Some methods by which to diagnose and remedy trace element imbalances within a flock.

The importance of assessing trace mineral status

Mineral nutrition is important in livestock production and should be considered as part of a whole farm approach to efficient production, particularly as farms move to optimise health and production. Optimising mineral balance ensures the sheep have sufficient mineral nutrition for their requirements which may improve fertility, immune function and body condition, and lower mortality rates; without overspending on unnecessary minerals, or introducing the risks associated with oversupply and toxicity (NRC 2007; Suttle 2010).

After first ensuring that feed intake, including, protein and energy needs are met, and infectious diseases are well managed, mineral provision can be investigated as a cause of poor health or sub-optimal production. Sheep production typically relies heavily on the utilisation of grazed and conserved grass, although some housed systems may rely on cereals. Irrespective of source, the main 'bulk' of the diet should ideally provide all the nutritional elements required. However, it is widely acknowledged that in many areas and on many farms forages and feedstuffs may over or undersupply various nutrients (AHVLA 2014; AFBI 2016; Clarkson and Kendall 2018), and the nutritional composition of pasture will also vary during the year and between years (Wiener and others 1978; Humann-Ziehank and others 2008; Clarkson and Kendall 2018). These variations, in addition to breed-related differences in mineral uptake and metabolism, mean that no two farms will be the same, providing an ideal opportunity for vets to work alongside farmers to establish an effective mineral programme.

Throughout the UK, the decision to provide mineral supplementation may be arbitrarily based on perceived nutritional deficiency, historic practice, or on analysis of grass, soil or animal parameters (Hession and others 2018; Clarkson 2019). Traditionally, mineral status may have been measured using an inconsistent and sporadic approach, with minimal measurements of blood parameters carried out as isolated tests. While this may provide useful information, if the goal is for optimal production and preventive nutrition, this alone may be inadequate for some trace elements. Mineral supplementation should be based on a thorough understanding of the total diet, considering if the forage analysis reflects the bioavailable supply of the mineral, and if the complete diet is balanced to mitigate the influence of interactions between minerals. The mineral supply can then be periodically reviewed alongside any management changes such as land improvements, changes in breed or stock numbers, or changes to the timing of key events (e.g., lambing) to maintain an optimal approach.

How to assess trace mineral status

Forage analysis

It is important to start by analysing the full range of pasture and conserved forage which constitute the animal's primary diet and subsequently, trace mineral intake. Forage mineral analysis confirms the total amount of element in the sample, and can detect minerals in forms which are not necessarily available to the sheep; examples include iron and manganese which are typically very low in availability versus the detected concentrations (Ammerman and others 1995). Forage analysis typically generates a visual report which compares the forage to 'typical' values, which do not relate to the animal's requirements. In simple terms, always use the stated amount and do not rely on the low, average and high descriptive graphs.

Soil and forage analysis is considerably advantageous to farmers over animal samples due to the ease of collection and lower analysis costs (Judson and McFarlane 1998). Soil cannot be used as an indicator of mineral issues in stock due to poor correlation between soil content, pasture uptake and, animal parameters (Ben-Shahar and Coe 1992; Judson and McFarlane 1998; Akhtar and others 2007; Dickson 2016). Forage analyses should be used as the management tool to identify which mineral element samples you may take from stock.

Forage analysis is necessary and cannot be considered as a 'one-off' activity. Seasonal changes alongside; rainfall, soil pH, species, strain and, maturity of the plant, are known to affect the mineral concentration of pastures (Dezfoulian and others 2012; SFC 2014; Clarkson and Kendall 2018). Trace minerals are also unevenly distributed in grasses, with leaves containing higher concentrations than stems (Suttle 2010). For these reasons it is important that forage samples are collected from each of the grazed areas and at varying times of year, allowing a picture of the nutrient provision specific to the farm to be built up over several years (Kendall and Bone 2019). Staggering the fields sampled to build a picture year on year can produce this effect without being too onerous as well as spreading the costs over time.

It is important to be aware that conserving forage can have an impact on mineral content and availability, especially through soil contamination which has been reported to negatively impact on animal production (Martens and others 2018), or ensiling increasing iron availability and its subsequent potential to negatively affect other mineral's absorption (Hansen and Spears 2009).

Animal analyses

Due to the potential for elemental interaction and genetic variation in livestock, dietary analysis should not be used in isolation but should be supported by analysis of animal parameters (Laven and Livesey 2004, 2005). Blood sampling carries advantages in that the samples are easily obtained, it is cost effective and widely available, but, the use of blood parameters alone provides an insight into the relatively immediate, short-term intake and status, and some parameters do not vary greatly unless the diet is extremely deficient or greatly in excess (Laven and Livesey 2009). Elemental serum or plasma concentrations may also represent the presence or absence of certain minerals, but not necessarily their functionality (Laven and others 2007). Blood enzyme function may give a better reflection of function, but may be influenced by unrelated internal or external factors such as stress or disease (Twomey and others 2005; Laven and others 2007). Despite these drawbacks, blood parameters remain useful to give a reflection of the animal's *current* status, especially when they are used year after year in conjunction with liver tissue and forage analysis (reviewed in Box 1).

Liver tissue samples are valuable for assessing the concentration of elements excreted via the hepatic system as they represent the longer-term mineral status and previous diet, not the present status of the animal, allowing perennial trends to be assessed (Laven and Livesey 2006). Liver tissue samples can be obtained through abattoir recovery, post-mortem recovery; especially from trauma culls, or via liver biopsy (technique shown in Box 2).

When to assess trace mineral status

Prime opportunities for animal assessment in grazing ewes are at post-weaning and pre-tupping. At these times both liver and blood can be collected to allow historic and present mineral status to be determined and necessary action taken ahead of key production periods, with sufficient time (up to 6 weeks) after a requirement is defined for the supplement to take full effect.

Sample sizes can be optimised based on the management of the flock to keep labour and costs at an optimum (discussed in Box 3). However, it is paramount that a random selection of healthy animals are used to give a fair representation, and that those with suspected clinical signs, other illnesses and, either the best individuals or 'poor-doers' are not specifically chosen.

For flocks where liver tissue sampling is undertaken by biopsy, and where there is a risk of parasitism with *Fasciola hepatica,* it is recommended that the random sample of ewes selected are pre-treated with a flukicide appropriate to the expected stage of parasitism at least three weeks prior to biopsy, to reduce the risk of haemorrhage in the sampled ewes.

A further key opportunity is presented pre-lambing, where trace element analysis can coincide with metabolic profiles for energy and protein. However, if ewes are being fed a supplementary diet at this time the data gathered may not be a true representation. At this stage, it is recommended that blood samples *only* are collected, as the risk from stress and complications from live liver biopsy are too high in the pregnant ewe. Also, due to the longer-term nature in the variation of hepatic concentrations, large changes would not be expected. Analysis can allow the immediate and medium-term status to be assessed alongside the efficacy of any changes implemented pre-tupping and any final adjustment, if required, ahead of lambing.

It is important to consider supplementation as part of a continuous cycle with analysis (Figure 1). Continued monitoring of animal status and forage provision is important, to allow the supplementation and management strategy to be best tailored to the animal's requirements. It may need several years of liver and forage data to fully build up a complete picture. Once this has been established, further analysis allows strategies to be reviewed over the years as both the grazing pasture and the sheep may change over time.

A case study of on farm mineral management

This case study is included as it gives a worked example of investigation, analysis and management changes, together with implementation challenges which may frequently be encountered by practitioners.

The flock comprised 450 Welsh mules, grazing 100 hectares in Wales. The ewes grazed mostly lowland pasture, with a short period spent on improved upland pasture around lambing time followed by housing for lambing. As with many sheep farms the trace mineral status of the flock was unknown and supplementation was routinely provided through a multi-element drench solution containing copper as copper sulphate, cobalt as cobalt sulphate, iodine as potassium iodide and selenium as sodium selenite, given twice, approximately two months and one month before tupping.

Initial status and actions

Investigation began by carrying out trace mineral analysis on a pooled grass sample (mixed ley with a high proportion of rye grasses, collected mid-summer 2018) taken from the two large areas grazed in rotation by the sheep, as well as conserved forage samples from baled silage (mixed ley with a high proportion of rye grasses). In addition, liver biopsies and blood samples were taken from eight randomly selected ewes pre-tupping and prior to the usual supplementation in summer 2018. All analyses were undertaken at the University of Nottingham Veterinary Nutritional Analysis (NUVetNA) service according to their commercial protocols.

The results from the pasture, conserved forage and animal samples indicated that the animals' copper status was being inhibited by elevated sulphur (3.73 g/kg DM) which can interfere with copper between 2-4 g/kg DM (NRC 2000, 2001, 2007), molybdenum (9.6 mg/kg DM) which can interfere with copper at concentrations >0.5 mg/kg DM; where sulphur is also elevated (Bone 2010; Axelson and others 2018; Laven 2018), and iron (782 mg/kg DM) where concentrations exceed 250 mg/kg DM (Bremner and others 1987; Prabowo and others 1988; Mullis and others 2003). Despite sufficient basal copper in the feeds (10.5 mg/kg DM) the presence of antagonists over these thresholds in combination with lowered liver copper status and blood parameters supported this conclusion.

Selenium concentration was low in both forage types (0.03 & 0.05 mg/kg DM) and insufficient to meet the expected requirements (0.04-0.5 mg/kg DM, NRC 2007). The liver selenium status was in the deficient range, as were the blood parameters. However, the plasma selenium was higher than the expected level of GSHPx suggesting the animals were less likely to require supplementation.

Cobalt concentrations were within sufficient range in the feeds (0.01-0.2, NRC, 2007) and the plasma cobalt status of the animals appeared above normal range. However, plasma vitamin B_{12}, a truer measure of functionality, was unavailable, which would have been useful as hepatic cobalt status was in the marginal-low range.

Both zinc and manganese in the feeds were sufficient to meet animal requirements (22-45 & 10-34 mg/kg DM respectively, NRC 2007), but appeared in the marginal-low/deficient ranges in the animal parameters.

From these initial results (Figure 2) it was recommended that a bolus to provide long-term (6 months), slow and continuous release of copper and selenium supplementation would be ideal (supplement choices are reviewed in Box 4), as the animals were out at pasture and this would provide a direct to animal dose to cover the grazing period. However, the farmer had administered the multi-element drench shortly after sampling and before the results were known. This raised concerns over copper toxicity if another source of copper was administered at this time. As a compromise, a further multi-component, trace element drench was given at scanning to counteract the expected decline in these elements.

Review for lambing

The flock was re-examined and re-sampled for blood only (n=8 sheep) three to four weeks prior to lambing as part of a metabolic profiling exercise to help, 'fine tune' the energy and protein supply for lambing. The ewes had been housed three weeks earlier (six weeks before lambing) and a small amount of concentrate feed had been introduced at a rate of 125g per lamb carried, commencing around the point of sampling. As the opportunity arose, liver tissue samples (n=8) were taken from new-born lambs that died from natural causes (e.g., dystocia, smothering, starvation etc.) for trace element analysis.

The two-drench approach was shown to be inadequate. The blood results showed that some of the ewes had worryingly low plasma copper concentrations and copper enzyme activity. Concern was raised with the farmer about the very real possibility of clinical swayback being observed in the lambs as a result. Plasma selenium was also low and although GSHPx had some results within normal range plasma selenium was lower than the expected level of GSHPx suggesting the animals required immediate supplementation.

As lambing had now commenced, the opportunity for intervention was limited; very close monitoring was carried out. Fortunately, no clinical cases of swayback were observed. However, lamb liver concentrations confirmed copper deficiency and both cobalt and selenium were towards the lower end of normal range. On the understanding that the sheep would be returning to the pasture for approximately 9-10 months, the decision was taken to supplement the ewes with copper and selenium, this time using a longer lasting (6 month) bolus, which also contained cobalt, as they left the shed and went back to pasture.

Summer review

In the summer of 2019, eight random ewes from the flock were again selected for investigation. The purpose was once more twofold: firstly, to evaluate the trace element status of the ewes to see if a similar intervention as in 2018 was required. Secondly, to monitor the response of the ewes to the trace element interventions already given. This year sampling was carried out a little earlier, one month post weaning, allowing more time to adjust and plan supplementation.

It was expected that the elements provided by the bolus would be utilised within ~6 months and then at post-weaning/pre-tupping in the autumn a similar bolus could be given to provide better supplementation for the pregnant ewes. However, the blood and liver results pre-tupping in 2019 were highly varied. Whilst some ewes were predictably deficient in copper, some demonstrated much higher liver copper concentrations and blood parameters. Although, many were within the normal range, some were also elevated which raised concerns that further copper supplementation could result in toxicity. As it was not possible to identify all the ewes at risk, copper supplementation was withheld at this time. Instead, to correct the low selenium concentrations in both liver, plasma and GSHPx, a selenium-only drench was recommended at pre-tupping and again at scanning. The farmer provided these as a selenium-cobalt combined drench.

Unfortunately, no analysis was conducted at pre-lambing in 2019 which meant that the efficacy of the drenches could not be checked.

Pre-tupping review

Returning to the farm pre-tupping in summer 2020 healthy, randomly selected ewes (n=8) were again analysed for their blood and liver status. The results continued to indicate that the selenium and copper status of the ewes was less than ideal. The hepatic copper status still had wide variation but bordering on normal range, with none of the sampled animals in the elevated range. The functional copper parameters in the blood were also lowered. Hepatic selenium remained in the deficient range with the blood parameters bordering marginal-low and bottom end of the normal range. Hepatic cobalt was noticed to be increasing year on year, likely due to its inclusion in the multi-element supplements, although this was of low concern. The advice was to provide a slow-release (6-9 month) bolus containing copper, selenium and cobalt administered one month before tupping, hopefully allowing a suitable supply of these elements during gestation and lactation with a 'washout period' prior to the next tupping season (2021).

Cost analysis

Typical laboratory costs vary with service and package but range between £20-40 per sample for forages and blood analysis, and from £20-50 per sample for tissue (2020 prices). Services are available from NUVetNA, Biobest, Axiom and Albion Laboratory Services as well as others. The post-tupping investigation included examination, blood, and tissue sampling for 8 ewes totalling £723.25. This figure included a veterinary examination and sampling fee, laboratory fees, parasitic screening, and consultation. Pre-lambing monitoring included examination and blood sampling costing £576.42. Including examination and sampling fees, laboratory fees, and a consultation fee.

Farmers would also 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 example, laboratory submission fees can be saved if farmers group together with neighbouring farms and send samples together. The cost of blood analysis per sample can be reduced if energy and protein analyses are not required or if just energy or just protein is required.

An unnecessary bolus could cost in the region of £1 per ewe which, if necessary, is likely to deliver a return, yet if not, it is an unnecessary cost on a potentially large scale. 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 increased veterinary costs. Conversely, under supplying concentrate feed pre-lambing is likely to result in reduced milk quality and quantity, with potential production and disease consequences for both ewes and lambs, as well as an increase the number of ewes with difficulties lambing, and, may potentially lead to pregnancy toxaemia and its consequences in some ewes.

Summary and conclusions

Managing trace mineral supplementation strategy on farm is multi-faceted. Imbalances of multiple elements simultaneously can make management problematic. The tendency of farmers to take action not based on analysis, in combination with the use of multi-element products can further complicate matters. Essentially, it is most important to balance the expectations of the animal's requirements with their input from *all* sources. Routine testing needs to be done in good time to highlight the potential problems at key stages in production and to allow ample time for their effective correction. Analysis on an ongoing basis to build a year-on-year picture also helps to inform decision making, to track, assess, and predict the likelihood of potential problems as well as allowing longer-term patterns to emerge. The data gathered from pasture analysis should be used to predict the risk of imbalance. However, before a supplemental strategy is devised and implemented it is important to consider the expected changes to management, especially in terms of housing, where different feeds may be used. Additionally, failure to account for any previous over-supplementation, along with the resulting increase in supply, can increase the risk of toxicity, especially for elements such as copper.

All supplementation strategies should be based on input and status data, although persuading farmers to move away from their traditional methods and strategies may pose challenges in practice. It is important to assess the efficacy of any strategy which can be done through simple checks on farm such as providing supplementation to one group of animals within the management group or to keep a small number of sentinels who receive no supplementation to see if there is a performance difference to the others.

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BOX 1: COMMON TRACE MINERAL ANALYTES AND WHAT THEY MEASURE

Analyte	Role
Glutathione peroxidase (GSHPx)	GSHPx is a medium-term (~6 weeks) measure of functional selenium through enzyme activity.
Plasma selenium	Plasma selenium concentrations reflect the short-term (<1 week) selenium content of the blood, it does not indicate if the selenium is biologically functional.
Liver selenium	Liver concentrations represent the longer-term (>6 months) selenium status. While short-term changes will not be reflected changes over several months to years indicate trends towards depletion or accumulation.
Caeruloplasmin (Cp)	Cp is a short-term (<1 week) measure of functional copper through the enzyme's activity, which can also be elevated during acute phase responses (Livesey and others, 2002). It is important to differentiate between the different assays for Cp activity and concentration; as Cp can be released in a non-copper containing form making Cp activity the more reliable measure. May give an indication of systemic interactions on functional copper.
Superoxide dismutase (SOD)	SOD is a medium-term (~6 weeks) measure of functional copper through enzyme activity. SOD does tend to vary with age and species. May give an indication of systemic interactions on functional copper.
Plasma copper	Plasma copper concentrations reflect the short-term (<1 week) copper content of the blood, it does not indicate if the copper is biologically functional.
Liver copper	Hepatic copper concentration is considered the best measure for total body copper status, due to the sequestering of copper in the hepatocytes (Strickland and others 2019), Liver concentrations represent the longer-term (>6 months) copper status. While short-term changes will not be reflected, changes over several months to years indicate trends towards depletion or accumulation.
Plasma cobalt	Cobalt concentrations can easily be raised by increased cobalt intakes and supplementation without necessarily being available for incorporation in vitamin B_{12}.
Vitamin B_{12}	Vitamin B_{12} concentration is a short-term measure of the functional role of cobalt.
Liver cobalt	Liver concentrations represent the longer-term (>6 months) cobalt status. While short-term changes will not be reflected changes over several months to years indicate trends towards depletion or accumulation.
Plasma zinc	Plasma zinc concentrations reflect the short-term zinc content of the blood. Plasma zinc levels may also be lowered by stress or infection.
Liver manganese	Liver concentrations represent the longer-term (>6 months) manganese status. While short-term changes will not be reflected changes over several months to years indicate trends towards depletion or accumulation.
Plasma inorganic iodine/ urine iodine	Plasma inorganic iodine and urine iodine both report iodine supply. Selenium status should always be assessed when considering iodine as selenium deficiency impairs iodine function (conversion of T4 to T3).

BOX 2: SHEEP LIVER BIOPSY TECHNIQUE

Restrain the sheep in left lateral recumbency. The site of incision is located at the 11th intercostal space on the right-hand side, at a distance of approximately one third ventral to the dorsal spinous processes. The area should be clipped and prepared aseptically (Figure a). A bulla of local anaesthetic (approximately 5ml) is infused into the skin and intercostal muscles. Once anaesthesia has taken effect make a small stab incision through the skin with a scalpel and then insert a 4mm trochar and cannula. This should be directed either transversely directly towards the opposite side of the sheep, or at an angle directly towards the contralateral elbow (Figure b). The trocar and cannular are advanced through the intercostal muscles and the diaphragm, at which point movement can be felt as the sheep breathes in and out. The trocar should then be removed before the cannular is advanced 1-2 cm further into the liver parenchyma and rotated slightly. A 10ml syringe should then be attached to the cannular and approximately 5-10ml of negative pressure applied to ensure tissue aspiration, before the swift withdrawal of the cannular and syringe together (Figure c). The liver tissue should then be deposited onto a sterile swab to remove the excess blood before transfer into a plain sterile container using forceps (Figure d).

a)  b) 

c)  d) 

Figures: a) Aseptic preparation of the site of incision; b) careful advancement of a 4mm trocar and cannular; c) negative pressure applied via syringe to aid aspiration of the liver tissue as the cannular is withdrawn; d) depositing the liver tissue in a sterile container after blotting on a sterile swab.

BOX 3: SELECTING A SUITABLE SAMPLE SIZE

When flocks are managed as separate management groups 4 sheep per group should be sufficient. When the flock are managed as a single group/flock this can be reduced to 6-8 individuals. Different individuals could be chosen for blood or liver sampling to further increase the range and reduce stress on individuals. However, paired sampling (liver tissue and blood samples from the same animal) can offer nuanced insights into mineral assimilation over the short, medium and longer term for the sampled animals.

BOX 4: BENEFITS AND DRAWBACKS OF DIFFERENT SUPPLEMENT TYPES

Supplement type	Benefits	Drawbacks
Pre-mixes in feed	Easy to administer. No animal handling required. Can be tailored by mineral and by management group.	Often formulated to textbook values not calculated to requirements. Not useful in grazed extensive settings. Miscalculations or inadequate mixing can cause toxicity problems.
Free-access minerals	Easy to administer. No animal handling required. Multi-element combinations available for different production stages. Inexpensive.	Unpredictable/ variable dose provided to each animal as some animals may consume a lot more than others. May exacerbate imbalance in the flock rather than remedy it. Palatable ingredients drive consumption not requirement. Unable to balance the mix to requirement.
Drenches	Intended to last 1-2 months. Animals receive an individual dose. Useful to correct deficiency. Available as single or multi-element mixes. Can be inexpensive.	Different elements have different response times and may last less than expected. Efficacy of the individual minerals varies in the mixture. Typically, Se lasts around 6 weeks more than the other minerals in drenches. Manufacturers tend to base stated duration on Se although the other minerals will likely have a shorter duration. For Co, Cu and Zn this may be only a few days. Animals must be tolerant of high concentrations to avoid toxicity. Administration requires handling.
Boluses	Intended to last several months. Animals receive an individual dose. Available as multi-element mixes. Slow-release lowers acute toxicity risk.	Length of activity is debated. Cu, Co, Zn and Iodine may last for the length of bolus dissolution. Infusion rate may be too low to correct severe deficiency. Administration requires handling and can take longer than drenching. Dissolution and absorption rates vary for different elements in the bolus. Elemental composition cannot be tailored.

		May be formulated at the maximum permissible limits (MPL), supply may be above this limit with the background supply.
Injections	Intended to last several months. Animals receive an individual dose. Useful for rapid remedy of a single mineral deficiency such as copper or selenium.	Administration requires handling and is time consuming. Risk of complications such as swelling and abscesses. Animals must be tolerant of high concentrations to avoid toxicity. Parenteral routes bypass normal homeostatic and absorptive controls which can predispose some animals to toxicity, especially for copper. Vitamin B_{12} injections are expected to have a short duration of <1 month. Not available for all elements.
Pasture dressing	Easy to administer. No animal handling required.	Unknown dose provided to each animal. Inaccurate distribution can lead to toxicity problems. Rainfall can wash away dressing. Efficacy is highly debated.

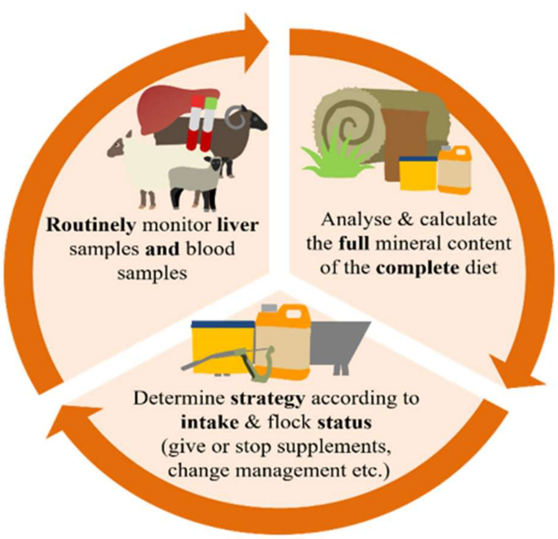

Figure 1: A diagram to show the cyclical nature of the supplemental cycle.

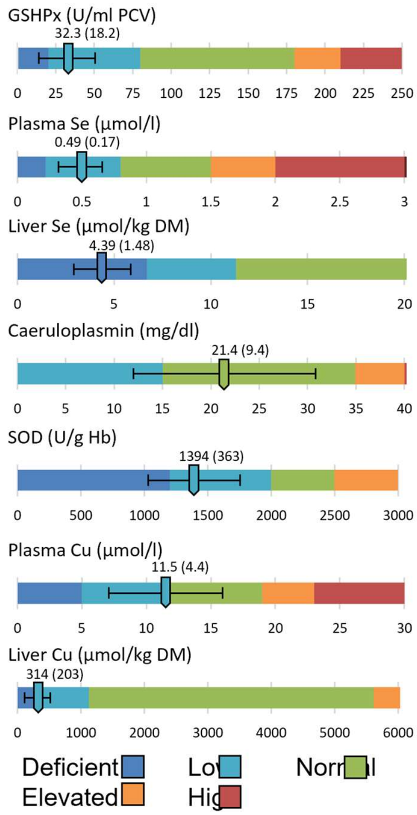

Figure 2: Mean blood and tissue results for animal parameters from 8 random ewes pre-tupping 2018. Arrow points to mean with mean value stated above. Parentheses and error bars show standard deviation. Interpretation scale based on ranges provided by NUVetNA laboratories.