Trace mineral testing in cattle: where and what to look for

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This article looks at what tests we can undertake to support our review of an animal’s trace mineral status and, more importantly, what tests are suitable and where we should be looking to diagnose issues associated with trace minerals.

Routinely, blood samples are taken to look at trace mineral levels and many conclusions are drawn from the results our laboratories return to us. It would be impossible to talk about all the trace minerals or potential complications within the scope of this article, but it is worth considering what we are testing for and the best samples to take to enable suitable interpretation.

Copper

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Copper is known to accumulate in the liver while blood levels are maintained by homeostasis within a narrow range. This means deficiency can be determined through blood testing, but identifying suboptimal levels or predicting when deficiency is likely to occur (before it does) will necessitate looking at the storage pool to see what is there.

This is also a good way of determining if excess copper is present (which the author has found to
be the case in a majority of total mixed ration herds sampled).

As such, liver biopsy is the preferred method for assessing copper status – ideally this is done in the live, healthy animal to enable a true reflection of what is occurring on the farm.

**Cobalt/vitamin B12**

Cobalt/vitamin B12 is also stored in the liver, so it is, therefore, a logical step if you are taking liver biopsy samples for copper to look at this also. Cobalt functions as an essential component of B12 (cobalamin). Normal levels in blood of cobalt/B12 are low, requiring very sensitive testing to detect suitably, therefore making liver tissue, with the largest concentrations, the preferred option for testing.

Correlation, however, between cobalt levels and active cobalamin is variable. Looking at methylmalonic acid (MMA) is potentially a better measure. MMA accumulates when methylmalonyl coenzyme A fails to be converted to succinate – a cobalamin-dependant process and a potential functional indicator of cobalt deficiency (Herdt et al, 2011). Measuring B12 in blood can result in erroneous results due to confounding factors, such as binders or the B12 not being released in the first place. As such, a non-blood-based test such as liver cobalt is advisable (Laven, 2006).

**Selenium**

Selenium can be measured either through the blood (whole or serum) or via liver samples. Whole blood contains both serum and erythrocyte forms and is around three times that seen in serum alone, although this ratio can vary as serum levels can alter, depending on sample handling, resulting in falsely elevated serum levels. The erythrocyte pool (mainly glutathione peroxidase; GPx) is a slowly changing one, so it’s a historical look at selenium levels, while the serum pool is a more rapidly changing state (Herdt et al, 2011). Although GPx is generally the test most used, it must be remembered sample handling and conditions under which it is analysed will modulate results. Liver samples are very useful, but the age of the animal needs to be considered as younger animals will have higher normal ranges due to differing requirements and partitioning (Herdt et al, 2011).

**Iodine**

Iodine can be confusing to look at, as when we think about what we are testing for, it doesn’t necessarily tell us what we want to know (Laven, 2006). Plasma inorganic iodine (PII) and thyroxine are the tests we commonly use – PII indicates the iodine intake levels as it is a representation of circulating iodine, while the thyroxine is a product of the activity of the thyroid gland, but, unfortunately, is an unstable measurement due to the many factors that can impact on it (parasitism, stage of lactation).
The mechanism of iodine uptake by the thyroid is similar to that used by the mammary gland, therefore milk iodine may be a better indicator than serum or blood. This is one of those scenarios where we need to use clinical acumen to try to decipher results and potentially use it in conjunction with response to supplementation trials or the observation of clinical issues (that is goitre, thyroid gland:bodyweight ratio, and weak calves).

Sample selection needs to be a choice based on the experience of the operator and also a knowledge of the limitations of the information obtained. That is, if you are looking to confirm a diagnosis of copper deficiency, then bloods may be appropriate and approximately six will suffice. However, if looking at serum selenium, 10 to 15 samples may be more appropriate due to herd variations and sample variability. If using GPx, however, six may be sufficient, but understanding this is a historical view. In the author’s opinion, assessment of trace elements status in otherwise healthy animals to ascertain adequacy rather than to confirm deficiency, necessitates liver biopsy samples. Sampling of animals going for culling is not ideal as they are being culled for a reason, therefore are not representative of the herd.

Liver biopsy

Make sure the animals are well fed prior to the procedure to ensure a full rumen, as this will ensure the liver is sitting within easy reach of the biopsy needle.

Performing a liver biopsy in the field is a relatively simple procedure with a minimum of 1g being required for analysis, which equates to around a 4cm core biopsy sample when using a large bore biopsy needle. Small cores can be obtained using Tru-Cut (two samples will usually suffice), but the author prefers a reusable instrument with an internal diameter of approximately 2mm to 3mm when looking to assess biochemistry (Vermunt, 2005).

The appropriate site for taking a liver biopsy sample (non-ultrasound guided) is on the right hand side of the cow and in the second to last intercostal space (11th space), approximately a hand
span down from the horizontal level of the transverse processes of the vertebrae. This site can also be identified by drawing an imaginary line from the right tuber coxae to the ipsilateral elbow, and where this intersects with the second to last intercostal space.

Appropriate surgical preparation of the site (shaving and aseptic preparation) should take place prior to performing the biopsy, with local anaesthetic infused under the skin and into the intercostal muscles. The target site should be towards the caudal part of the space due to the major blood vessels that pass close to the caudal borders of the ribs.

When making the stab incision through the skin, it is useful to pull the skin caudally at the time of the stab incision and when first introducing the biopsy needle, as when it is withdrawn on completion, the skin incision and needle hole will be offset, thus reducing the likelihood of a pneumothorax occurring.

Following the stab incision, the needle is then inserted and advanced with two distinct pressure points/layers to be penetrated – the intercostal muscles and the muscular diaphragm. Once through the diaphragm, the trocar can be removed and the needle directed towards the contralateral elbow and advanced gently with a rotating motion along the axis of the needle, to allow it to cut through the liver tissue, which will have a distinct “gritty” feel to it.

Slight suction should then be applied using a 10ml syringe (approximately 2ml to 3ml negative pressure) as the needle is withdrawn to retain the sample within the needle.

Once the needle has been withdrawn, the sample can then be expressed on to a clean/sterile gauze swab, allowing any excess blood to drain away before being placed into a small, sealable container and appropriately labelled. If histopathology is required, then fixative should also be added at this time.

The skin incision is left open and sprayed with a suitable topical product for wounds and, as long as the animal sampled is adequately up to date with clostridial vaccinations, then prophylactic antibiotics are not indicated. If clostridial vaccinations are not up to date then a single therapeutic dose of penicillin should be administered following the procedure.

Complications are not common provided suitable training of the operator has taken place and due care is taken, although the potential for complications does mean this should be discussed with owners before undertaking the procedure and appropriate steps taken.
The liver biopsy needle and trochar.

If a hepatic blood vessel is penetrated, blood will flow from the needle when the trocar is removed. Redirecting the needle will see this cease and, unless a haemorrhagic tendency is present, then this should not result in subsequent issues. Pneumothorax can occur, but this is usually only one sided and will often resolve within a few hours (indicated by coughing post-procedure).

The procedure can be performed in any crush that allows good access to the right side of the animal being biopsied. It is important to instruct the owners to ensure the animals are well fed prior to performing the procedure to ensure a full rumen, as this will ensure the liver is sitting well to the right of the animal and within easy reach of the biopsy needle.

Animals that have been standing off feed, waiting for you to arrive for a number of hours (such as just after milking), can be particularly hard to obtain quality samples from. If everything goes to plan, a single sample will provide you with approximately 4cm of core sample without the need to insert the needle a second time.

**Summary**

Trace mineral testing is useful to identify potential production-limiting elements, but must be done
with full understanding of the limitations of the tests and a knowledge of how to interpret the results. Not enough is known of where production limiting and adequate levels are for the trace minerals, and although deficiency and toxicity levels have been identified for some time, these are very different entities that would benefit from further work.

Liver biopsy samples are not difficult to obtain with adequate training and should be included alongside bloods when looking at routine assessments of trace minerals, as blood alone is not enough.

References