TRACE MINERALS MANAGEMENT IN CATTLE

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Introduction

Trace minerals are known to be essential in animal nutrition because they play a major role in health, reproduction and productivity. The most important trace elements in cattle are selenium (Se), copper (Cu), zinc (Zn), iodine (I), cobalt (Co), manganese (Mn) and iron (Fe).

The first question we are faced with is whether trace minerals constitute a real difficulty in cattle herds. The answer is not unique but depends on the productivity level of the animals, on the concentration of each trace mineral in the ration and the interactions between these, and on the objectives of the management of these herds. Indeed, selection of highly productive animals, as well in dairy than beef breeds, goes hand in hand with the increase of their needs and is not always associated with an increase of their dry matter intake capacity, prescribing a greater concentration of the different components of their ration, of whose trace minerals. Moreover, some trace minerals could be deficient in the ration while others could be present at a plethoric or even toxic level. Multiple deficiencies are the rule and it is not always easy to incriminate one of them in particular in case of decreased herd performances. Lastly, the recommended trace minerals supply will vary according to whether the objective is only to prevent the deficiency symptoms or also to optimize health, welfare, reproduction and productivity of the animals, as well as the quality of the food (milk and meat) produced.
Anyhow, numerous papers have described the negative effects of trace elements deficiencies on cattle's health (Graham, 1991). In Europe, trace elements deficiencies are nowadays widely spread in cattle. A recent study (Guyot et al., 2009) presented the Belgian epidemiology of Se, Zn, Cu and I deficiencies in dairy (mainly Holstein) and beef (mainly Belgian Blue) herds with health problems, compared to healthy herds. As expected, lower blood trace elements concentrations were found in unhealthy herds. These results in Belgian herds corroborated those previously found in Ireland (Mee and Rogers, 1994) and France (Enjalbert et al., 2006) and can probably be extrapolated to most European countries.

The second question concerns the reasons of such a situation. First of all, soils in Europe are most often deficient and nearly never replenished in a series of trace minerals. Secondly, with the advent of monocultures such as corn and ryegrass silages, rations of cattle are less and less varied and well balanced. Last but not least, pecuniary difficulties of many farmers incite them to decrease production costs by suppressing the minerals in the ration of their animals.

In Spain, there seems to be scarce information about trace minerals concentrations in soils and their extraction by the crops. A positive correlation was found between the liver Cu of cattle at slaughterhouse and the density of pig-rearing units, due to pig slurry spread on pastures grazed by cattle (Lopez-Alonso, 2012). By consent of the same author, "the fact that in most cases these mineral deficiencies are subclinical or have not clear symptoms, and that the clinical veterinaries have no experience to deal with them, makes diagnosis difficult".

Consequently, the objective of this paper is to provide to cattle practitioners the tools to correctly manage trace minerals deficiencies and excesses in cattle herds. The first step will be the appraisal of the trace minerals status through different clinical symptoms, afterwards confirmed by selected laboratory tests. It will then be explained how to correct the revealed anomalies, taking into account the speculation (dairy or beef) and the respective operating procedures of herds management.

Clinical observations linked to trace minerals troubles

A series of elements must first make the practitioner a priori suspicious about trace minerals troubles in cattle herds: ration mainly based on poor forages like corn silage, straw and too fibrous hay, lack of minerals distribution, soil ingestion and contamination of forages by soil rich in Fe. Recent research suggests that acid conditions occurring during fermentations of silage greatly increase the bioavailability of soil Fe in cattle (x 80!).

Clinically, there is a wide range of symptoms associated with trace element deficiencies but clinical signs are rarely pathognomonic. However, some are more suggestive than others: embryonic and fetal death, abortions, premature calving (I), maladaptation of calves to extra-uterine life in general (Se and I) and stillbirths, weak and cold calves, congenital muscular rigidity, different causes of dysphagia (macroglossy, congenital myopathy of the tongue), respiratory distress syndrome and greater susceptibility to infectious diseases in particular, anemia (Cu, Co -> Vit. B12), myopathy and cardiomyopathy (Se), diarrhea (Cu, Co), skin diseases like mange, ringworm, digital dermatitis and footrot (Zn, Cu, I), pica not due to energy, protein, sodium or phosphorus deficiency, mastitis and increased bulk milk cell counts (Se), retained placenta and metritis (Se), fat cow and downer cow syndromes (I, Se, Co).

Laboratory tests

As previously mentioned, laboratory analyses are absolutely necessary to confirm the diagnosis, at least when no previous information is available. Most of the time, deficiencies are multiple (Guyot et al., 2009) and associated with some trace minerals in excess and it is important to know which elements are deficient or excessive in order to adequately supplement the animals and avoid competition between minerals. In our practice, we generally analyze forages produced in the farm as well as blood and/or milk of healthy animals. We never use urine and hair analyses that are not sufficiently reliable but it occasionally occurs that we sample tissues, mainly liver, at necropsy or at slaughterhouse. Indeed, liver biopsy is often considered as complicated and risked for living patients and farmers are generally reluctant to this procedure.

Forages

We commit these analyses to a specialized lab in The Netherlands (Blgg, à compléter). The complete analysis including dry matter (DM), energy and protein components, pH, minerals (with chloride and sulphur also allowing the calculation of the DCAB) and trace minerals (Cu, Zn, Mn, Se, I, Co, Fe, Mo) costs about 120.00 €. It is very useful, mainly for grass silages whose composition could vary a lot, depending on the type of soil, on grass maturity stage at mowing time and on the harvest conditions (contamination by soil). In our conditions, it is not rare to find grass silages with a Fe concentration of several grams/kg, the maximum ever reached being 3.5 g/kg DM. Other trace minerals are habitually very deficient, with the exception of Mn.

Blood

Blood samples are easily taken and the modern techniques of assay are accurate, precise, sensitive and fast enough to make a good diagnosis. The inductively coupled plasma – mass spectroscopy (ICP-MS) has recently proven its efficacy at this point of view. Most of the time, blood is centrifuged and plasma or serum are used for ICP-MS assays. Except for Cu (plasma > serum), plasma and serum values are similar. Blood is generally an early indicator of the deficiency phase as serum/plasma represents the transport pool of the mineral.

Blood sampling

It is not the goal to remind how to correctly sample blood, but to focus on the misinterpretation that can be made from wrong samples. Everything must be done **to avoid haemolysis** that can falsely increase Se, Cu and Zn plasma/serum concentrations. The **delay** between sampling and analysis may also lead to false results. A long delay will increase the risk of haemolysis but also the diffusion of Se from erythrocytes (contained in glutathione-peroxidase (GPX)) to serum/plasma. The activity of enzymes (e.g. GPX or superoxide dismutase (SOD)) may also decrease with time. Finally, some tubes with rubber plugs may contain Zn, leading to a false increase of plasma/serum Zn concentration. **Specific plastic tubes**, without rubber and free of trace of minerals should be used.

Animals

Diagnosis of trace elements status in cattle should be done at the herd level because this is clearly a herd puzzle. The diagnosis consists in evaluating the nutritional and/or functional status of the herd and **healthy animals** should be sampled. Their number depends on the deficiency prevalence and the intra-herd variation of trace elements concentration. According to different authors, this number fluctuates **from 7 to 15** (Herdt, 2000; Herdt et al., 2000; Guyot and Rollin, 2007a). Animals to be sampled must be selected to form a homogeneous group in the target population. Analysis can be made **individually or by pool**. The pool has the advantage to be less expensive. It is accurate if the number of selected animals is sufficient (about 15 animals) and if the group of sampled animals is homogenous. One pool per target population is necessary. Nevertheless, pool is effective on plasmatic Se, Zn, Cu, I, but is useless for determining activity of GPX in erythrocytes, SOD or thyroid hormones. While using pool, attention must be paid in the interpretation of results if outliers (heterogeneous group) are present in the sampled population. In general, if the group is homogeneous, an “all-or-nothing” rule is applied as the majority of sampled animals (>70%) are either deficient or have a correct status (Guyot et al., 2009).

Variation sources

Haemolysis is a huge source of variation as it artificially increases plasmatic Cu, Zn and Se concentrations. Indeed, GPX and SOD in the red blood cells contain great amounts of these trace elements. **Inflammation** and diseases will secondarily increase plasmatic Cu concentration, as the inflammatory protein Cu-containing ceruloplasmine increases in case of inflammation. Concomitantly, a decreased plasmatic Zn concentration will occur while inflammation is present. Inflammation has also an impact on Se metabolism and thyroid hormones (e.g. Euthyroid Sick Syndrome). **Physiological factors**, such as gestation, range of lactation, age (calves *versus* dams) may influence blood trace elements levels as well. **Teat-dipping with iodophors** interferes with the diagnosis of I deficiency (in milk or plasma) by trans-cutaneous absorption of I. The injection of **some anthelmintic drugs** (closantel, nitroxynil) that contain I will also increase the plasmatic I for a quite long period (several months). The concentration of Se in milk can vary depending on the **form of Se** given in the ration, with more Se transferred to colostrum or milk when cows are fed selenomethionine compared to Na-Selenite. Moreover, at equal dosage of Se in the feed, plasmatic Se and, in a lesser extent, the activity of GPX, reach higher levels if selenomethionine is given in the ration, compared to Na-Selenite. The correlation between GPX and plasmatic Se is well described in the literature; however this correlation may change according to the form of Se as well. In any form of Se, following an oral supplementation, plasmatic Se will gradually increase to reach a steady state between 30-60 days, while GPX will continue to progressively increase at least up to 120 days (Guyot et al., 2007d).

Analyses and interpretation

Evaluating trace mineral status on blood or animal's tissues is subject to some limitations and considerations. Two different ways are often offered: determining **nutritional status** (e.g. plasmatic or hepatic concentration of the trace elements) or **functional status** (e.g. thyroid hormones, GPX, SOD). Even the **thresholds** are often polemic. Table 1 proposes different thresholds for assessing nutritional and functional status of the major trace elements Se, Zn, Cu and I. According to Fig.1, they have been chosen considering the concentration of trace elements needed in order to optimize health, welfare, food quality and productivity and not just avoiding clinical diseases or barely reaching N.R.C. requirements (NRC, 2000 and 2001). Beside this, blood and tissues (e.g. liver) represent different pools of trace elements in the body. When dietary intakes are not sufficient, the pools of storage and transport will first decrease, but they must be depleted before seeing dysfunction and disease (Fig.2). Ideally for the diagnosis, the storage pool must be sampled but in practice, it is not easily accessible.

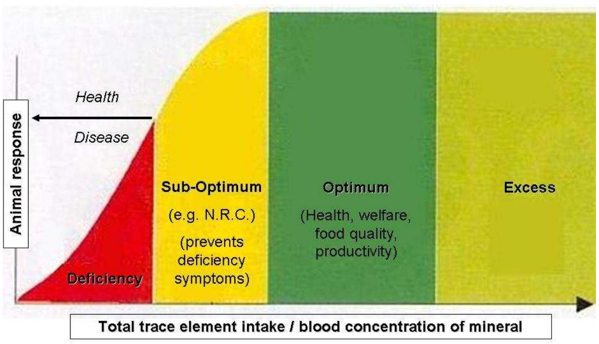

Fig.1: Threshold for determination of correct trace element status in cattle (adapted from Chung, 2003).

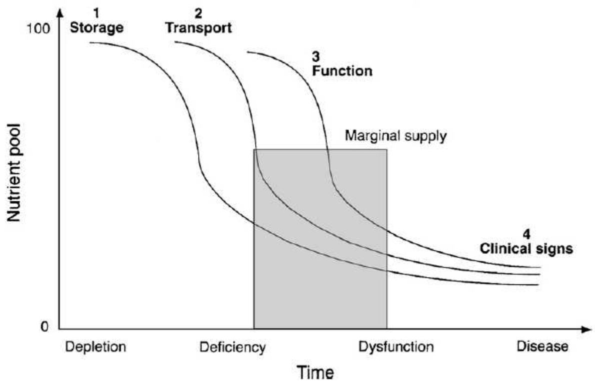

Fig.2: Representation of the kinetic of the pools of trace elements, according to the mineral level (from Underwood & Suttle, 1999).

Table 1: Threshold for plasma zinc (Zn), plasma copper (Cu), activity of glutathione peroxidase in erythrocytes (GPX), plasma selenium (Se), plasma thyroxine (T4), plasmatic inorganic iodine (PII) in blood and selenium (Se) and iodine (I) in milk for adult cattle (Herdt et al., 2000; Guyot et al., 2007d; Guyot and Rollin, 2007a; Guyot et al., 2009; Guyot et al., 2011).

Herd status	Zn (µmol/L)	Cu (µmol/L)	GPX (IU/gHb)	Se (µg/L)	T4 (nmol/L)	PII (µg/L)	Milk Se (µg/L)	Milk I (µg/L)
Adequate	14-21	13-18	220-600	80-140	30-120	45-650	> 50	80-500
Marginal	8-14	8-13	75-220	65-80	20-30	15-45	30-50	30-80
Deficient	< 8	< 8	< 75	< 65	< 20	< 15	< 30	< 30

Bulk milk can give an idea of the trace mineral status of a lactating herd. It is very easy to sample but limited to certain elements (Se, I).

Specific diagnosis for Se

Serum/plasma Se is good indicator of dietary intake. Plasma Se is a nutritional marker that quickly increases (within 2-6 days) after oral supplementation (Ellis et al., 1997).

For functional marker, or long-term marker, the assay of GPX in red blood cells is often used. Because the Se-containing GPX is formed at the time of erythrocyte's development, the GPX activity allows determining Se status of the 100-120 previous days (mean half-life of red blood cells). As Se is well transferred from dam to calf, the cow in late gestation will show lower Se plasma concentration. After calving, Se levels will progressively increase in the dam. In calves, a higher proportion of Se is in the GPX rather than the plasma, so the GPX activities are higher and plasma Se lower, compared to their dams (Guyot et al., 2011). I supplementation has an impact on Se metabolism, with low Se status while animals are fed supplementary I, compared with I-deficient diets (Pavlata et al., 2005; Guyot et al., 2011). Finally, milk Se can also be assessed but is principally influenced by the form of Se given in the ration (Guyot et al., 2007d).

Specific diagnosis for Cu

For Cu, the storage pool is the liver that also protects the animal against a possible toxicity. It reflects the long-term availability of dietary Cu. A decreased liver Cu concentration is an early marker of insufficient Cu dietary intake. The most sensitive way to assess Cu deficiency is therefore the liver biopsy, even if practitioners and farmers are unenthusiastic to this method of sampling. Instead, plasma/serum Cu is more often used. The transport pool is constituted by the blood and, in a certain extent, also the functional pool. In fact, ceruloplasmin, a protein of inflammation produced by the liver, contains about 80% of the Cu in the blood. Plasma Cu assessment seems to be better than serum because a variable amount of Cu may be captive in the clot. There is a curvilinear relationship between liver and blood Cu concentration. However, hepatic Cu concentration must fall drastically before a decreased blood Cu concentration can be seen (Underwood and Suttle, 1999). Plasma Cu is thus a late (long-term) marker of Cu deficiency but remains useful at the herd level, with at least 10-15 animals sampled per herd (Herdt, 2000). The SOD is a Cu-containing enzyme sometimes used in determining Cu status but it is very delicate to interpret.

Specific diagnosis for Zn

The storage pool for Zn is not well-defined, making the evaluation of Zn status tricky. Even if hepatic Zn concentration decreases after a long inadequate Zn dietary intake, the liver Zn is not a good means for assessing Zn status in cattle. Thus, the plasma/serum Zn concentration is preferred because it is reduced in case of Zn deficiency. However, there might be dysfunctions (e.g. reduced feed efficiency) before observing a decreased plasma Zn concentration. A low plasma Zn concentration is indicative of a deficiency while a normal serum Zn does not necessarily exclude a deficiency (Underwood and Suttle, 1999).

Specific diagnosis for I

I deficiency can also be assessed by nutritional or functional markers. For the nutritional ones, the most used is the plasmatic inorganic iodine (PII). The PII reflects the dietary I intake and its concentration fluctuates very quickly according to the I fed in the ration. For functional markers, the hypothalamus-pituitary-thyroid axis is investigated with bovine thyroid stimulating hormone (bTSH) and total thyroid hormones (thyroxine T4 or tri-iodo-thyronine T3) (Guyot et al., 2011). Huge variations can physiologically exist (pregnancy, circadian rhythm, birth, age) and are important to be included in the interpretation of the status. Clinical pathology laboratories can easily assay thyroid hormones while there is no laboratory able to measure bTSH at the moment. bTSH, T4 and T3 are usually taken for diagnosing hypothyroidism that originates mainly from I deficiency in cattle (Guyot et al., 2007b,c). Total blood I is constituted with PII and I from thyroid hormones, setting the interpretation of a short-term and long-term marker not obvious. Milk I can be used for assessing herd I status. It also varies quickly with dietary I, but in a lesser extent than PII. On the field, weighing thyroid gland in stillborn or goitrous calves is useful to appreciate I status. The maximal weight of the gland has been determined as follows (Hernandez et al., 1972): Y (weight of thyroid gland in grams) = $0.348 \times Z^{0.944}$ (Z being the body weight, in kg).

Specific diagnosis for Co
Co status in ruminants can be determined by Co or vitamin B12 blood concentration. However, the assessment of Co status in ruminants is complicated and poorly reliable with routine laboratory assays (Underwood and Suttle, 1999: Herdt et al., 2000).

Minerals supplementation
Correcting trace minerals deficiencies could be achieved in a direct or indirect way. The indirect correction consists in the spreading of fertilizers containing the needed trace minerals. It is very interesting for Se that is transformed by plants in selenomethionine. However,

Table 2: Recommended supply of Cu, Zn, Mn, Fe, Co, I and Se in cattle rations.

Trace mineral	**Recommended (ppm)**	**Maximum (ppm)**	**Toxicity (ppm)**
Cu	**9 - 18**	40	> 40
Zn	**43 - 73**	300 (1.000 if ZnO)	> 500 (700 : calf)
Mn	**25 - 40**	1.000	> 1.000
Fe	**12 - 22**	1.000 (500 = better)	> 1.000
Co	**0,11 - 0,35**	10	30
I	**0,35 – 3,5**	10	10
Se	**0,3-0,5**	1	10 mg/kg BW (acute) 5 - 40 ppm (chronic)

Conclusions
Trace elements deficiencies are frequent in Europe in cattle. As there are no pathognomic signs, clinical pathology diagnosis is necessary. Sampling at a herd-level increases the reliability of the diagnosis, if samples are taken from a homogeneous group of healthy

animals. Blood is most often used because of its higher feasibility on the field. But the storage pool, that is the most effective one for assessing mineral status, is not always represented by the blood. Specific herd thresholds for determining trace elements status in cattle must include health, welfare, food quality and productivity.

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