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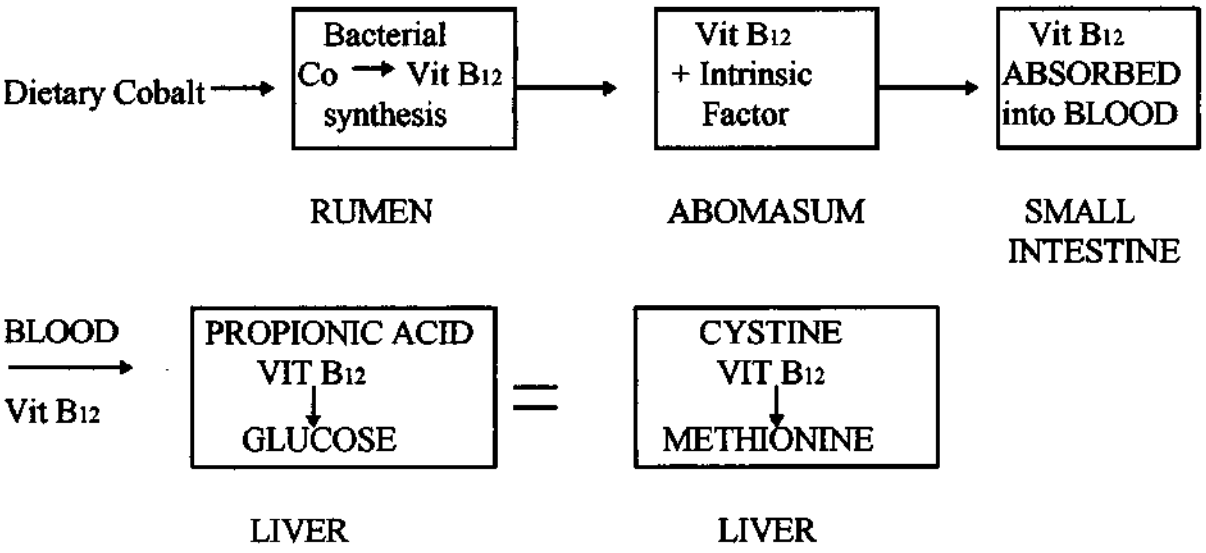
INTRODUCTION

Problems with the supply of essential trace-elements to dairy cows and followers in the United Kingdom can be restricted to copper, cobalt, selenium and iodine. There is also some evidence that a supply of zinc in excess of an animal's metabolic requirement may have a prophylactic effect. Animal welfare issues are also a topical subject in current systems. Work in the USA suggests that the supplementation with chromium at levels well above metabolic requirements can reduce stress in the animals.

However, as far as the UK is concerned, deficiencies of cobalt, selenium and copper are the most important economically.

Cobalt

The effect of a cobalt deficiency on ruminant animals is well understood. The biochemical pathways have been elucidated and are summarised below.



The significance of the above is that cobalt supplementation is only effective if the cobalt is available in the rumen for the bacterial synthetic process. An adequate cobalt intake (0.2mg/KgDM) through grass or rumen digestible feeds will supply the bacteria with enough cobalt to meet the animals B_{12} requirement. If the dairy cow receives less than this amount then a deficiency could occur. The major problem with cobalt in cattle is in diagnosing the cobalt status of the animal. Tests for cobalt status have been based on the assessment of the concentration of Vitamin B_{12} in the blood. Work with several species of ruminants - sheep, goats and cattle, has shown that the blood Vitamin B_{12} concentration correlates well with the cobalt status in sheep and goats, but not with cattle (Carlos et al 1987). This problem is an assay problem. In cattle blood there can be incomplete release of Vitamin B_{12} from its binding proteins (underestimates the B_{12} concentration) or there is an excess of "Vit B_{12}" in the blood (presence of metabolically inactive B_{12} analogues). The elevation of methyl malonic acid in blood does indicate a B_{12} deficiency, but this analytical method does not lend itself to routine analysis.

Therefore for cobalt what can we say about the need for supplementation in cattle? If you have sheep that suffer from cobalt deficiency (Pine) then cattle grazing the same pasture are also likely to be cobalt deficient. Work carried out by ADAS (Leeds) in the 1970's indicated that in areas of the Yorkshire dales where pasture was recognised as being deficient in cobalt, supplementation of dairy cows with 10mCo/KgDM resulted in an increased milk yield and an improvement in milk quality (Fat). This supplementation was given through the concentrate and became a means of recycling the cobalt. The dung returned to the pasture the excess cobalt originally in the compound feed and, as is well recognised, the net result was a transfer of cobalt from soil to plant to animal.

Fertilisation of low cobalt pastures with cobalt is therefore a simple though initially expensive method of curing the problem. As long as a regular daily supply of cobalt is given in the feed of the animal then the bacteria should be able to synthesise enough Vit B_{12} to meet its needs. The fashion of chelated minerals which are designed to bypass the rumen means that cobalt is not an element that requires to be "expensively" chelated. The cattle most at risk are those grazing low cobalt pastures and not receiving any supplementary feed. In these instances a slow-release form of cobalt is required such as the original Permaco bolus, All-Trace or Cosecure. These will all provide cobalt within the rumen for as long as they are present. Oral drenches such as the cobalt in wormers or in Pardevit or Liqui-Thrive will only give a very short-term response as the cobalt will "pass out" of the rumen within 2 to 3 days.

Cattle grazed on low cobalt pastures, but treated with cobalt will have an improved growth performance.

Selenium

The essential role of selenium was first identified in the 1960's and its metabolic function has been largely elucidated. The requirement for selenium in the enzyme glutathione peroxidase and its synergistic role with Vitamin E in removing toxic

peroxides from the body has been understood for some time. The more recent work by John Arthur (Arthur & Beckett, 1994) at the Rowett Institute in identifying a role for selenium in Thyroxine synthesis has explained the ill thrift condition found in lambs which responds to selenium supplementation but not to Vitamin E. The amount of selenium required by ruminants is similar to cobalt (0.1 to 0.2mg/KgDM) and also, like cobalt, it can be applied to pastures because there is a transfer from soil to plant to animal. However the effect of Se fertilisation is only 3 to 6 months unlike the 3 to 5years achievable with cobalt. Selenium is also a highly toxic element and a level of 3mg/KgDM is recognised to cause an acute toxicity. Excessive fertilisation of the pasture cannot therefore be carried out. Another factor in the transfer of selenium from soil to herbage is that sulphur and selenium are directly competitive and with the increasing advocacy of sulphur fertilisation of pasture, this could lead to a reduction in the selenium concentration of the grass.

Animals consuming low selenium grass are at risk of white muscle disease at the severest deficiency, but difficulty at calving and birth of weak calves are also related to selenium deficiency. The role of selenium in the thyroid production of Thyroxine can also be a cause of poor growth rates in selenium deficient animals. The other situations that arise are that animals with a low selenium/Vit E status, while appearing perfectly normal, could be susceptible to other agents administered to the animals (trigger factors) such as some antibiotics or a large sudden intake of polyunsaturated fatty acids (spring turnout, bypass lipids). Reduced resistance to infections can also be related to selenium status as the killing effect of the phagocytes is reduced in a deficient condition and this explains the connection between selenium status and some incidences of mastitis and pneumonias.

The animal with an adequate selenium status will therefore be healthier and more able to resist infection such as mastitis. Therefore while there may be no overt response to selenium supplementation there is a need to keep the animal at an adequate level in case of a future challenge. Selenium supplementation is unlike cobalt in that the selenium is incorporated into metallo-enzymes. Because of its similar chemistry to sulphur, the behaviour and metabolism of the two elements are very similar, with selenium toxicity basically being the replacement of sulphur by selenium in critical molecules in the body. The incorporation of selenium into glutathione peroxidase and hence its activation means that diagnosis of selenium status through erythrocyte GSHPx activity is a very reliable tool. As the proteins containing selenium turnover, the selenium is made available for reutilisation and therefore unlike cobalt there can be a reasonable duration of effect after a single dose of the element. However excess selenium cannot be given as the margin between an adequate dose and a toxic dose is small.

The selenium incorporated into wormers or given in oral solutions can give a boost to the body's selenium status for a 3 to 4 week period. Chelated selenium or probably selenomethione/selenocystine are organic forms that enable the selenium to be absorbed and utilised as the amino acids. Some of the inorganic oral doses will be utilised by the rumen bacteria and also converted into the seleno amino acids of the bacteria which will be subsequently digested and absorbed. Selenium released by rumen boluses will also be utilised by the bacteria and hence supply the animal with a steady supply of the seleno amino acids so long as the bolus is releasing selenium into

the rumen environment. The effect of the bolus selenium can also be present up to 2 to 3 months after the bolus has gone, due to the previously mentioned recycling of the seleno amino acids

There is therefore nothing magical about chelated selenium. The inclusion of the seleno amino acids in the diet and their bypass of the rumen is merely speeding up the biochemistry of the rumen bacteria. If they are given an adequate supply of selenium (0.2mg/KgDM) they will synthesise the animals needs. The seleno amino acids are however more readily utilised than the inorganic selenium used in injections such as Deposel (long lasting) and Vitenium (short lasting) but these injections show that the body's metabolism can convert inorganic selenium into a useable organic form.

Selenium therefore is an important element in dairy cattle. The animals most at risk will be followers or cows on a low compound feed intake where grass or silage is virtually the sole feed available.

Copper

So far I have discussed cobalt and selenium and indicated that we have identified their pathways and functions. Copper has been recognised as an essential trace element since the late 1920's/early 1930's and has been studied and examined in more detail than any other trace element. This work has enabled us to identify a large number of copper dependant enzymes whose activity is "lost" when copper is not present in the structure. Ruminant animals have been found to readily show symptoms of clinical copper deficiency which range from what appears to be minor changes (i.e. coat colour) to severe problems (i.e. swayback in sheep). The effect of molybdenum and sulphur on copper "availability" has also been identified and termed secondary or induced copper deficiency, with animals showing symptoms of swayback, coat colour changes, poor growth and infertility being some of the symptoms.

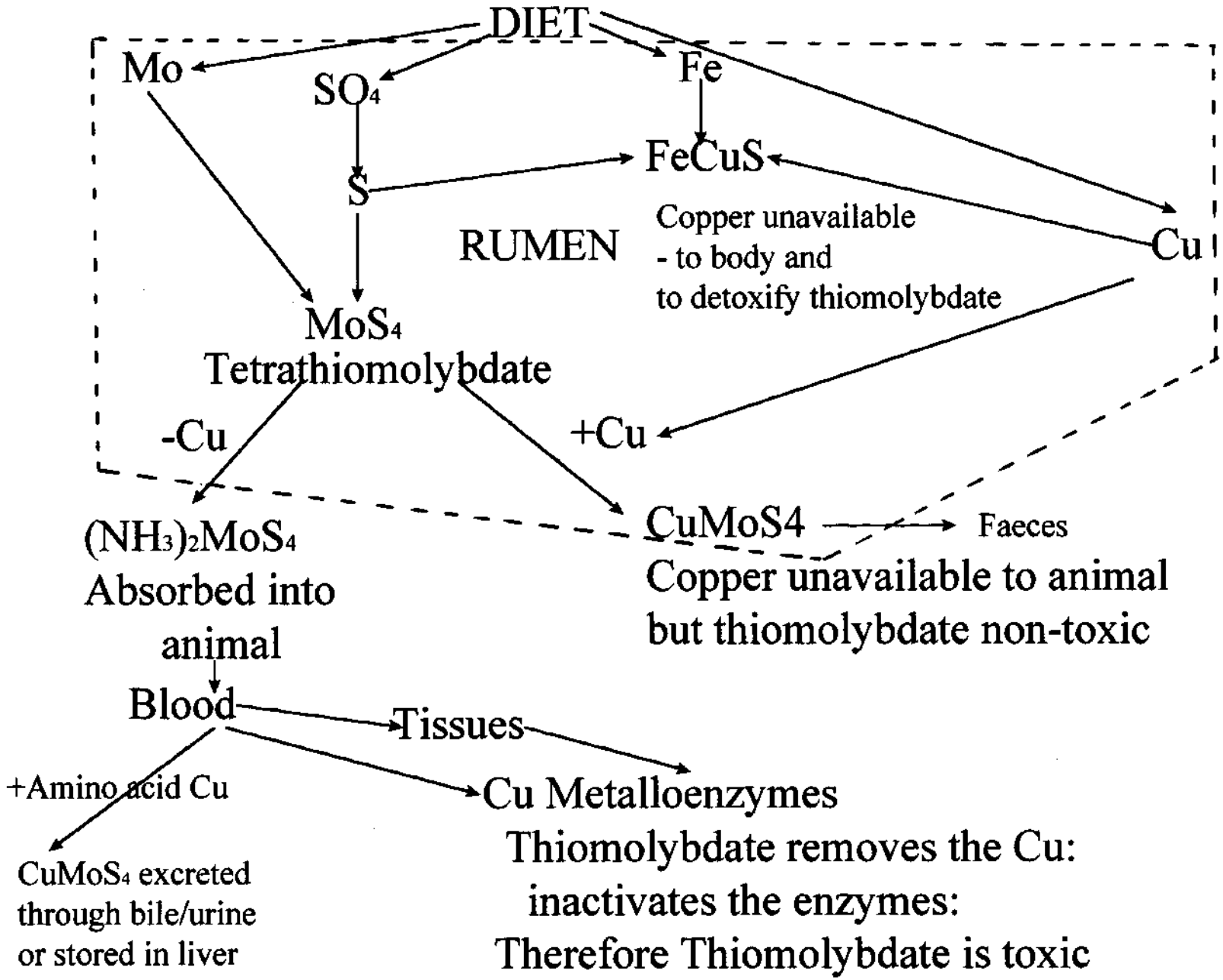
In the past few years, however, the work of Phillipo et al (1985) at the Rowett has shown that the inclusion of iron at a level of 250 mg/KgDM into a cattle diet which had no molybdenum in it resulted in animals with severe hypocupraemia but no clinical copper deficiency symptoms. By all the current diagnoses of copper deficiency these animals had low blood coppers and low liver coppers. They were however perfectly normal and continued in this hypocupraemic state for up to 15 months before showing the only clinical symptom which was a loss of appetite. This was corrected by the injection of a few mg of copper. However, if these high iron-fed copper depleted cattle were given 2 to 3 mg Mo/KgDM in their diet, the clinical symptoms described as copper deficiency occurred with coat colour changes being the overt sign of the effect. Similarly, when molybdenum but not iron was included in the diet, the animals developed clinical symptoms. Work at the University of Leeds has recently confirmed this finding in sheep and cattle.

What is the significance of these findings? Animals can become hypocupraemic without developing clinical copper deficiency, while animals with normal copper levels (blood and liver) can show clinical symptoms. The problem is not a lack of copper for metabolic purposes, but rather a toxicity of molybdenum. When

molybdenum is included in the diet the rumen bacteria use it along with sulphur to synthesise compounds known as thiomolybdates (di, Mo S2, tri, Mo S3, tetra , Mo S4). Until recently thiomolybdate was regarded as the compound that bound with copper to form copperthiomolybdate which rendered the copper unavailable to the animal and hence induced the deficiency. The inclusion of iron along with sulphur also forms a Cu/Fe/S complex which renders the copper unavailable to the animal, but in this instance there are no symptoms of clinical copper deficiency. Therefore, what is happening in the animal? The thiomolybdate is synthesised in the rumen and, if there are no copper ions for it to bind to, it associates with ammonia to form ammonium thiomolybdate which is then absorbed through the rumen wall into the bloodstream. If copper ions are available in the rumen then copper thiomolybdate will be formed and excreted in the faeces. The presence of high iron levels in the rumen will bind copper along with sulphur and reduce the quantity of copper ions available to bind to the thiomolybdate. Once the thiomolybdate is absorbed into the blood stream, it has a higher affinity for copper than any other ion. In blood the copper that is available to react with the thiomolybdate is the amino acid bound copper. This amino acid bound copper is only a small pool and constitutes only 1 to 2% of the total blood copper. The thiomolybdate will "remove" this copper from the amino acids (if it is present) but if thiomolybdate is present in excess of this amino acid copper pool, the thiomolybdate will then find other sources of copper in the blood and tissues as it has a stronger affinity constant for this copper. The molecules which get attacked by the thiomolybdate are the copper enzymes, the net result of this attack being that the enzymes lose their activity as though they were "poisoned" by the thiomolybdate. When the copper enzymes lose their activity the result is that the animal shows overt signs such as coat colour changes, poor growth and infertility, these being the symptoms described as clinical copper deficiency. What we actually can have are animals whose copper status is still "adequate", but whose copper enzymes are non-functional, not through a lack of copper, but rather a poisoning by thiomolybdate. Clinical copper deficiency is, therefore, a toxicity of molybdenum in the presence of sulphur and this is exacerbated by increasing levels of iron in the rumen. (Fig. 1)

Ruminant animals will thrive quite happily on a diet of less that 1mg/KgDM so long as there is **no** molybdenum in that diet. The stated copper requirements of 5mg/KgDM for sheep and 10mg/KgDM for cattle with these concentrations being increased in the presence of an increased molybdenum (>2mg/KgDM) and sulphur (>2g/KgDM) content. What is now clear is that the significant feature of copper is **not** the copper available for absorption and metabolic utilisation in the body, but rather the copper ions available in the rumen to bind to and hence neutralise the thiomolybdates or, alternatively, the second line of defence against the thiomolybdate toxicity is the blood amino acid associated copper. The cure for clinical copper deficiency is to supply the animal with copper over and above its metabolic requirements in order to completely "neutralise" the thiomolybdates.

How do we supply this copper to the animal? In grass the copper will be mainly present as part of the protein fraction, as it will also be in other dietary protein sources. As the protein is digested in the rumen, the copper will be made available. The supplementary in-feed copper source is generally either copper sulphate or "chelated" copper predominantly as a proteinate. Copper associated with sulphur will


DIET
Mo
SO4
Fe
FeCuS
S
RUMEN
Copper unavailable
- to body and
to detoxify thiomolybdate
Cu
MoS4
Tetrathiomolybdate
-Cu
+Cu
(NH3)2MoS4
Absorbed into
animal
CuMoS4
Faeces
Copper unavailable to animal
but thiomolybdate non-toxic
Blood
Tissues
+Amino acid Cu
Cu Metalloenzymes
CuMoS4 excreted
through bile/urine
or stored in liver
Thiomolybdate removes the Cu:
inactivates the enzymes:
Therefore Thiomolybdate is toxic

be utilised in the rumen by the bacteria to form copper sulphides which, in the presence of iron, will form the iron/copper/sulphur complexes and thus limit the copper available in the rumen to react with thiomolybdate. The copper proteinates that pass into the abomasum and beyond will be digested and their copper absorbed by metallothionein in the small intestine. The copper from copper sulphate that escapes the rumen, but which is not associated with iron or sulphide will be equally well absorbed as the copper from the proteinates. Any difference in "availability" is likely to be due to the amount escaping the rumen chemistry. Copper oxide needles and copper oxide powder are inert in the rumen and require the acid environment of the abomasum to release the copper and render it absorbable. Copper injections are given subcutaneously and release copper from the site of the injection at varying rates. All of these treatments, with the exception of some of the copper from copper sulphate, are supplying their copper into the blood stream. They therefore replenish the plasma amino acid copper pool which is then available to neutralise any absorbed thiomolybdate i.e. the second line of defence against the thiomolybdate. The copper supplied by these treatments is finite and if the thiomolybdate exceeds the supply then clinical symptoms will occur. Similarly, the greater the amount of thiomolybdate that is absorbed, the faster this will use up the amino acid copper and thus reduce the time for which the treatments will be effective. The only supplement to refuse copper ions directly into the rumen and hence neutralise the thiomolybdates before they are absorbed, is the soluble glass bolus Cosecure. All the copper released is ionised and available to react. With high iron levels some will obviously go to form iron/copper/ sulphur compounds, but the ready reactivity of its copper ions will result in the detoxification of the thiomolybdate in the rumen. In order to produce a similar effect through the blood, the copper must be constantly available to replenish the amino acid copper pool and hence chelated copper will give a response if included in total mixed rations or fed several times per day. The use of oral drenches of chelated minerals (high mineral yeasts) will give a temporary respite. The bulk of the dose - some 90% passes through the intestine within 2 to 3 days. The copper that is absorbed will associate with the amino acids and remove the thiomolybdate being absorbed.

Now that we know the theory, what is the response in the animal? Infertility in the dairy cow is a common problem on many farms. The work at the University of Leeds has identified that in some 2,000 cattle bloods less than 5% had low blood copper levels that would have been diagnosed as copper deficient (MacKenzie & Telfer 1996) However, we have developed a blood diagnosis based on the ratio of a copper enzyme's activity (caeruloplasmin) to total plasma copper that indicates that greater than 70% of these animals would be expected to respond to copper therapy despite having blood copper levels currently regarded as "normal". When these "normal" animals were suitably treated with copper then a response in fertility has been seen by the veterinary surgeon/farmer. For example, in our own dairy herd we have found that although blood coppers have ranged from 14 to 17μmol/l (normal range 12 to 23 μmol/l) we have seen a significant increase in calving interval from 376 to 396 days in animals not treated with Cosecure. These findings and the ability to diagnose animals that will respond to copper therapy should result in an improvement in overall fertility in many dairy herds.

Conclusion

I have tried to indicate the responses that can be expected in cattle when the correct trace element supplementation is given. This paper has concentrated on the three trace elements of major importance in cattle and has, I hope, identified areas where we can control the supply and maximise the return.

Elements such as zinc and chromium are currently topical and are likely to be promoted for their "beneficial" effects above metabolic requirements. Claims made for zinc include effects on lameness, footrot, broken mouth, mastitis and prevention of facial eczema. Some of these have been reasonably well established whilst others are still more tentative. The absorption of this extra zinc is related to copper and is controlled by the metallothionein. However, zinc, unlike copper, is not "stored" in the body and there is a limit to the amount of "extra" zinc that can be absorbed. Therefore, further work is required to confirm the benefits of supplemental zinc.

The trace elements included in an animal's diet are an essential component of its overall nutritional requirements. Continued scientific investigation is a necessary ingredient of producing the optimum result. The work on copper deficiency illustrates how an understanding of the problem can aid in the treatment of the animal. With copper the next step is to work out the quantities of iron, copper, molybdenum and sulphur in the diet and how they interact in order to produce an equation that can indicate the "risk" of clinical symptoms occurring in the animal.

The area of trace element supplementation still has some secrets to reveal and will continue to be a fascinating area of research that will have benefits for animal production.

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