RESEARCH TOPIC REVIEW: Management of trace elements and vitamins in organic ruminant livestock nutrition in the context of the whole farm system

Author: M.J.Tame BSc PhD

Scope and Objectives of the Research Topic Review.
This review collates and summarises the organic research into trace elements and vitamins in organic ruminant livestock, including that commissioned by Defra and that undertaken elsewhere and incorporates field experiences in the conclusions.

It covers the role of trace elements and vitamins in animal health, their requirements (where known), and the extent and effect on health of known trace element and vitamin deficiencies. It also considers strategies for improving the level of trace elements available to ruminant livestock in forages as well as the role of supplements in various forms in treating established deficiencies. Gaps in our knowledge will be highlighted.

Role of trace minerals and vitamins in animal health.
The role of trace elements is well covered in the Compendium of Animal Health and Welfare in Organic Farming produced by the Organic Livestock Group, (2000) and by Underwood and Suttle (1999). In the hope that it might be more helpful in understanding the role of trace elements the approach taken here will be to consider their role in a number of ruminant disease/health problems.

Many trace elements have very specific but often multiple roles, for example selenium. It has been known for a considerable time to be necessary for growth and fertility in animals and for the prevention of a variety of disease conditions. More recently it has been established to form an integral part of a number of enzymes (selenoproteins) most of which function as antioxidants in the cellular cytoplasm in a range of situations. Others are involved in the conversion of Tetraiodothyronine (T4) to Triiodothyronine (T3), the active form of the hormone thyroxin (a list of some of these enzymes and their function is given by Underwood & Suttle, (1999).

As well as individual trace elements having several functions several trace minerals and vitamins may be involved in a single function. For example it is well established that vitamins A and E as well as selenium, zinc and copper are all involved in immune function (see Meglia 2004)

Rather than consider the role of each trace element and vitamin it is felt that a review would be more helpful to consider the role of trace elements and vitamins in some of the more common problems encountered in ruminant livestock

Immune competence and susceptibility to disease
It is well established that the incidence of infectious diseases such as mastitis and endometritis in dairy cows increases during the periparturient period with mastitis being the most common (Eberhart 1986, Grohn & Rajala-Schultz 2000 and Smith & Hogan 2001). Mismanagement of the dry period, particularly the transition period can predispose to both metabolic and infectious diseases (Ostergaard & Sorenson 1998, Rukkwamsuk et al 1999). In addition, a deficiency of micronutrients notably vitamins A and E, selenium and zinc have been associated with increased incidence of diseases (Kellog 1990, Hemingway 1999, Weiss 2002). The dry period should be used to assess the animals' trace element status and correct any deficits

While the importance of vitamins and trace minerals is established it has also been noted by a number of workers (Johnston & Chew 1984, Oldham et al. 1991, Smith et al 1997, Meglia 2004) that levels of vitamin A and E and selenium and zinc vary with time in blood and milk, dropping towards the end of the dry period and around calving recovering during lactation. Indeed, Meglia (2004) showed that vitamin A and E levels dropped to critical levels in a proportion of the animals studied. It has also been noted that levels of glutathione peroxidase decrease at parturition and recover slowly during lactation (Tame 1995). Part of the explanation for this may be that colostrum contains more vitamins and selenium than milk (Goff & Stabel 1990, Underwood & Suttle 1999, Meglia 2004), part may be due to the decrease in DMI in the last 7-10 days prior to calving and part may be due to increased stress levels around calving.

Vitamin E is the most important fat soluble anti-oxidant (Putnam & Comben 1987) and forms an integral part of the cell membrane and functions to protect the poly-unsaturated fatty acid (PUFA) component from damage by reactive oxygen species (Rice & Kennedy 1988) . The most important PUFA is arachidonic acid as it can be metabolised into compounds that are important in mediating the tissue response to pathogen invasion (Astrosi 1986, 1989). While vitamin E acts as an antioxidant in the cell membrane, selenium, as part of the group of enzymes known as glutathione peroxidase, is present within the cell and functions as probably the body's most important intracellular anti-oxidant (Diplock 1981 & Erskine 1993).

Pathogen invasion of the mammary gland triggers a cascading influx of a number of different white blood cells (Craven & Williams 1985, Meglia 2004). This is followed by phagocytosis of the pathogens and results in a respiratory burst producing peroxides that kill the pathogen but are also potentially dangerous to the cell and surrounding tissues. At this point any deficit in antioxidant activity will result in damage to surrounding tissues and either prolonged or further infection. Unless removed by antioxidants reactive oxygen species (ROS) such peroxides cause significant cell damage that, in extreme cases, can result in conditions such as white muscle disease in calves.

0ther studies have shown that measures designed to increase blood selenium and vitamin E levels in the dry cow resulted in lower levels of infection and a more rapid clear-up of infection in the immediate post calving period (Erskine 1998).

More recently, the importance of selenium in immune function has been established (see G S Meglia 2004) in dairy cattle.

Scaletti et al (2001) have shown that supplementing diets with 20 ppm copper reduces the severity of mastitis following *E. coli* challenge compared with diets containing only 7 ppm. A separate study showed that heifers fed 20 ppm supplemental copper from 84 days pre to 107 days post-partum compared with heifers fed no supplemental copper had fewer infected quarters (Harmon & Torre 1994). Again, copper is an integral part of a number of proteins that act as antioxidants (see Weiss 2005).

Zinc has also been shown to influence the incidence of infection and the recovery rate in both mastitis and somatic cell counts (see Weiss 2005). Tomlinson et al (2002) summarized the results of 12 experiments and reported an overall significant reduction in somatic cell count (SCC) when diets were supplemented with 200 mg zinc per day. On the other hand Whitaker et al (1997) reported no significant effect on infection rate, new infections clinical mastitis and SCC when similar levels of zinc supplementation either in the form of zinc proteinate or inorganic zinc or a combination of the two was used. Harmon (1998) has suggested that part of the effect of zinc may be related to skin integrity as a deficiency of zinc is known to be weaken skin and other stratified epithelia and the keratin lining of the mammary glad may physically trap bacteria and prevent migration into the mammary gland. It is also known to be involved in acute phase response to infection and inflammation (Prasad 1979)

A study by Boland et al (1996) using a supplement containing copper, selenium and zinc as the bioplex showed that the reduction in SCC increased with time of lactation reaching as much as 52% by week 12.

Questions have also been raised about the role of zinc in resistance to internal parasites in sheep. However, studies have shown that appetite and growth rate are affected before there is any effect on resistance to internal parasites (Underwood and Suttle 1999) and it is possible that the effect is mediated through maintenance of the integrity of the cells lining the intestine and lungs.

Trace element status in ruminant livestock
Establishing the trace element status of ruminants is not straightforward. For example blood copper levels are not a good indicator of copper status as not all the copper is available to the animal. Levels can be influenced by antagonist such as molybdenum, sulphate, infection, trauma and stage of production (Puls, 1990), nor are serum copper levels highly correlated to liver copper levels (Clarke et al 1993). For example, cattle with low plasma copper levels had adequate liver copper levels (Mulryan and Mason 1992). Swenson (1998) and Boland (2003) have also shown that liver copper levels vary with stage in the production cycle declining during the pre-parturient period reaching its lowest at calving and increasing post calving. It is also known that plasma levels of selenium change dropping markedly in the peri-parturient period and increasing slowly during lactation (Tame 1995).

Clearly the timing of any assessment in relation to the production cycle is very important.

Trace element requirements
For the UK trace element requirements of ruminant livestock are given in "Mineral, trace element and vitamin allowances for ruminant livestock" in ARC (1980) for the UK and in NRC Nutrient requirements of Dairy Cattle (2001) for the USA. The UK levels were drawn up in 1980 and some requirements, are based on rather sparse information. For the UK more recent suggestions have been made by Alderman (1993) and McDonald et al. (1995) for trace minerals and these differ from those given in ARC (1980). The recommendations for livestock in the USA is perhaps more up to date being revised in 2001 (NRC 2001). However, Weiss (2002) suggests that these should be regarded as minimum requirements – as they do not included safety margins. The groups in both Ohio (Weiss, 2002) and Kentucky (Scaletti 1999) are offering very similar suggestions for feeding levels as follows:

Vitamin E	1,000 IU/day for dry cows 500IU/day for lactating cows
Selenium	0.3 ppm
Copper	20 ppm
Zinc	40-60

Though Weiss' group goes on to note that as the dry matter intake falls in the last 2 weeks pre calving it will be necessary to increase the level per kg feed dry matter in order to maintain the 1,000 IU/day intake.

While the UK recommendations do generally include a safety margin they do not take account of the presence of antagonists. For example vitamin E requirements increase in the presence of high levels of unsaturated fats and oils. Copper uptake is inhibited particularly by molybdenum but also by sulphur and to a lesser extent by iron (Underwood and Suttle 1999). There are also interactions between minerals for example high levels of calcium in the feed inhibit the uptake of zinc. It is also established that higher levels of copper are required in the presence of high levels of zinc and that animals under stress require higher levels of copper and zinc. There is also some evidence from personal observations (Tame 2006 and Measures 2006) that some deficits are associated with soil types, for example, deficiencies of selenium and zinc in livestock appear to be more common in animals foraging herbage from thin soils overlying chalk and limestone.

Such interactions make it very difficult to set general requirements. Indeed, a more helpful approach would be to consider each situation in its own right.

Beck (1962) in a study in Australia states that copper deficiency diseases in ruminants occur when copper levels in pasture are 3 ppm or less and that above 6 ppm they did not occur. 3-6 ppm was regarded as marginal.

<u>The extent of trace mineral deficiencies in organic ruminant livestock.</u>
Whilst the author is not aware of any specific scientific studies in this area there is much accepted

information. For example, it is very well established that livestock grazed on the Somerset Levels rapidly exhibit the characteristic signs of severe copper deficiency as a result of very high molybdenum levels in the forages eaten.

Another example is parts of Derbyshire and Devon where livestock exhibit signs of iodine deficiency. In parts of Devon the iodine deficiency is complicated by selenium deficiencies.

Other examples are areas such as some parts of Dorset, Wiltshire, Gloucestershire and Lincolnshire where forages are growing on thin soils over either chalk or limestone brash where there appear to be deficiencies of selenium, copper and zinc (Tame 2003 personal observations).

Measures has also commented (Measures 2007) that there are deficiencies of selenium and cobalt in livestock grazing in the Herefordshire/Welsh border region.

It is also generally accepted that there are deficiencies of selenium and copper in the forage grown under conventional conditions over large areas of the country. There does not appear to be comparable information with regard to forages grown under organic husbandry.

It is possible that trace mineral deficiencies may be less prevalent in organic systems for a number of reasons. In many cases production levels are lower so the daily trace mineral requirements are likely to be lower. In addition, higher levels of forages are usually fed and a higher proportion of the forages are from permanent pasture a proportion of which may contain herbs with a higher content of trace minerals (see below).

<u>Sources of trace elements and vitamins</u>

There is a considerable amount of data collected over a long period of time on the trace element levels in forages, particularly from ryegrass based swards. However, the over-ridding feature of this data is the very wide range of values for each element. A tenfold or greater range between the lowest and highest values is not uncommon making the interpretation of the data extremely difficult. The problem is that no reference is made to the stage of growth, the ratio of leaf to stem to flower/seed head, at which the samples were harvested or to the weather conditions or light intensity under which the samples were harvested. It has been known for some time that values are different in leaf, stem and seed and even differ between young and old leaves (see review of Selenium in Higher Plants, Terry et al, 2000, and Goodwin-Jones 2007). Thus a silage sample from a sward cut prior to heading will be expected to have a higher selenium level than a sward cut when it is in flower. Many organic farmers take only one cut of silage and to ensure that they have a sufficient quantity leave the sward to "bulk up". This usually means that the grasses have made the switch from vegetative to reproductive growth and this will have consequences for selenium content. There is also evidence that the form in which selenium is available has a strong influence on the level of selenium in the plant (Terry et al, 2000).

It is likely that variations in the content of other trace minerals will be dependent on stage of growth, ratio of leaf to stem, weather conditions etc, may also apply to other trace minerals.

While most of the trace elements are essential to plants this is not true of all of them. There is still a controversy as to whether selenium is essential to plants or not though it is know that there is a group of plants which appear to actively accumulate selenium (Parker and Page 1994).

There is also much anecdotal evidence that herbs such as ribgrass (Plantago lanceolata), yarrow (Achillea millefolium) and dandelion (Taraxacum officinale) etc. contain much higher levels of trace minerals. However, the same comments apply as made above. An additional comment is that there are only a small number of studies to date in which grasses, legumes and "pasture weeds" (herbs) have been grown under the same conditions and analysed for minerals and trace elements (Harrington et al., 2006, van Eckeren et al. 2006, Weller and Bowling, 2001). The Harrington study was conducted in New Zealand and samples were taken mid-summer though there is no comment about growth stage. Levels of both macro and micronutrients were compared between ryegrass, Yorkshire fog, white clover, chicory, narrow leaved plantain, broad-leaved dock, and dandelion. The chicory and narrow leaved plantain had significantly higher levels of phosphorus, sulphur and sodium, chicory also had higher levels of magnesium and the narrow leaved plantain had higher levels of calcium. Dandelion had significantly higher levels of phosphorus magnesium and sodium. With regard to micro-nutrients Chicory had significantly higher levels of copper, zinc and boron, while narrow leaved plantain had higher levels of copper and cobalt. Dandelion had higher levels of copper, zinc and boron,. Yorkshire fog had a significantly higher content of molybdenum at a level likely to interfere with copper absorption.

In the same study the levels of selenium were highest in white clover but also higher in the “weeds” than in the ryegrass though the difference between the “weeds” and ryegrass was not significant. This was despite the pasture having been treated with 27 kg/ha of Selenium Ultra the previous autumn.

The van Eckeren et al (2006) study compared ryegrass, white clover, chicory and plantain and was conducted on two different soil types, sandy and clay, though plantain was only grown on the sandy soils. This study showed that calcium levels were very much higher in white clover and chicory on both soils and in plantain on the sandy soils. Sodium was higher in both chicory and plantain on the sandy soils and in chicory on the clay soils. Of the trace minerals copper was much higher in the chicory on both soils types than in the grass or clover. Zinc was higher in the chicory and the plantain than in grass and clover on the sandy soils and higher in the chicory on the clay soils. On the clay soils the chicory had higher contents of both cobalt and selenium than either grass or clover. There were no significant differences on the sandy soils.

Weller and Bowling (2001) have also shown that there were differences in the major minerals confirming that white clover and chicory had much higher levels of calcium, magnesium and sodium and ribgrass plantain had much higher levels of calcium and magnesium. Surprisingly, timothy was lower than ryegrass in both calcium and sodium. Clover was also lower in phosphorus and potassium. However, they also highlighted a significant complication by showing that the level of sodium varied through the season with, for example, ryegrass having levels of 997 mg/kg DM in May-June but 1,302 mg/kg DM in July-October. The level of sodium in the ribgrass plantain, on the other hand varied in the opposite direction with 1,262 mg/kg DM in May - June but only 799 mg/kg DM in July - October. The levels in chicory were 7,193 mg/kg DM in May - June and 13, 885 mg/kg DM in July October. There also appeared to be a seasonal variation in magnesium content in ribgrass plantain. It is also well known that rapidly growing ryegrasses have lower levels of magnesium that can under some conditions result in staggers in cattle. Variations such as these only serve to highlight the difficulty of interpreting other data in the scientific literature. At present mineral analyses rarely note the stage of growth of the sward when the sample was taken. The question has to be asked as to whether there are seasonal variations in any of the other trace element levels in any or all of the herbs commonly present in swards? A further complication is that while chicory may be a very good source of sodium compared with ryegrass and ribgrass it’s proportion in the sward was quite low compared with either ryegrass or ribgrass but increased as the grass seed rate of the ryegrass decreased. The proportion of ribgrass also increased as the grass seed rate decreased though the clover content remained fairly constant. The chicory, ribgrass and clover were all sown at a constant seed rate while that of the grass varied.

There is also some data on mineral composition of chicory and plantain by the group at the Appalachian Farming Systems Research Centre in the USA (Matt et al. 2003) citing both their work and that of others (Thomas et al 1952, Jung et al 1996 and Belesky et al 1999) which shows that chicory and plantain generally have higher levels of calcium, magnesium, copper and zinc than orchard grass though there is a wide variation between the studies.

Clearly this is a complicated area. The level of minerals not only varies from one plant species to another but also appears to depend on soil type and growth stage.

Strategies for assessing trace element status in ruminants

Clearly, in the light of the earlier findings detailed above, great care needs to be taken when assessing the trace element status of ruminant livestock. It appears that marginal trace element status often does not result in clearly defined symptoms.

Perhaps the best starting point for organic livestock is the forage, both grazed and conserved, as this has to provide a minimum of 60% of the daily dry matter intake in organic systems and in most cases provides considerably more. However, note should be made of the stage of growth, weather conditions, time of year, composition of the sward etc as these are all likely to affect trace mineral levels. Such analyses should give a good indication as to whether the animals is likely to have adequate, marginal or deficient levels of a particular trace element.

Assessment of the ruminants trace element status is much more difficult. Account needs to be taken of its point in the production cycle, the level of stress imposed on the animal, the choice of analysis, the level of trace element antagonists as well as other trace elements in the feed and indeed the nature of any supplementary feeds used.

At present the main strategy for improving trace element status in ruminants is to analyse the forages and possibly other feeds and to seek a derogation from the appropriate Sector Body to feed a tailor made mineral supplement or administer an injection of the appropriate trace element to overcome any deficits highlighted by the analyses. However, this should be regarded as a short-term measure as it is a largely conventional approach and not in keeping with organic philosophy. This poses the question of how can we devise a more “natural and sustainable” strategy?

Range of plant species

The information highlighted above, even though it is very incomplete, suggests that one approach would be the inclusion of a much wider range of plant species in the grazing and conservation swards used for ruminant feed. A number of seed houses are now offering seed mixes which include a range of grasses, clovers, trefoils and herbs. While these mixes are generally significantly more expensive and take longer to establish the indications are that they are much longer lasting. If they also result in better trace element status in the livestock it is likely that the extra cost will be more than offset by a combination of less frequent re-seeding and better animal health. However, before any reliable recommendations can be made we need to understand much more about how the level of each trace element varies in different parts of the plant as well as how it varies with stage of growth through the season. If quantitative recommendations are to be made account will need to be taken of the proportions of the various herbage plants within the sward or great care will need to be taken when sampling to ensure that the sample is truly representative of the sward.

Soil management

Another strategy being adopted is to “re-balance” the soil and the micro nutrient availability by altering the cation exchange capacity of the soil by the addition of the appropriate compounds after detailed analysis of the soils and the adoption of the “Albrecht” system of soil analysis or by improving “soil health” by improving the biological activity of the soils by a combination of soil aeration and increasing the soil microbial activity or by a combination of both. There is little scientific evidence to support either of these strategies with regard to crop production let alone the uptake or transfer of trace elements.

There are a number of organizations, notably Soil Science Ltd, Groundhog Nutrition and Field Services that advocate the application of trace mineral supplements at very low levels to the soils to “rebalance” the mineral levels in those soils.

Claims are made that such strategies are effective and result in more productive swards/crops though there appears to be no peer reviewed studies that provide supporting data. There are however, many testaments from “satisfied customers”.

Effect of chemical/biological form of trace minerals on uptake
While the approach described above may be useful for some trace elements the indications are that it may only be partially effective for others, for example selenium. As pointed out above there is still controversy as to whether selenium is an essential element for plants or not (Terry et al., 2000). If it is not essential it will only be present in the plant as a contaminant and, consequently, may not reflect the soil selenium level. In addition, there is evidence that the form in which the selenium is presented to the animals is important. Grace et al, 1997 showed the there was no significant difference between the effects of giving the selenium either in the form of a selenium bolus or a barium selenate injection. Both forms resulted in a much higher blood selenium level as well as a much higher level in the milk from the treated animals. However, Givens et al, (2004) administered selenium at three different levels to dairy cows either in the form of sodium selenite, a chelated selenium product or a selenium yeast product. Significant increases were observed for all three sources though the selenium yeast gave a much greater response. They also commented that the efficiency with which the selenium was transferred to milk was much greater for the selenium yeast source. Unfortunately, blood selenium levels were not measured in this study but it seems reasonable to assume, in the light of the Grace et al, (1997) study, that blood selenium levels were improved.

It should be noted here that the uptake of selenium by plants appeared to be much higher if the selenium was in the form of selenate rather than selenite. This poses the question as to whether there may a similar difference in the uptake in ruminants.

Supplementation of trace elements in organic livestock is most commonly made through feed, rumen boluses and free access mineral blocks. There is also some use of trace element injections, particularly where are local inhibitory factors such as soil molybdenum affecting copper uptake. Injections have the disadvantage that the rumen is by-passed and consequently rumen microbes may not receive an adequate supply of trace elements for their critically important functions.

Conclusions.
There is a wealth of information on the vital role of trace minerals in ruminant health. This information gives some reasonable indications of the levels required by dairy and beef animals to maintain good health though there is much less information available for sheep. There is also a wealth of information available on the role played by trace elements in the major diseases of ruminants, particularly mastitis and the problem of high somatic cell counts. There is also information available on the effects of trace mineral supplementation on fertility and production.

This information should be used by organic farmers and their advisors to assess trace mineral status of animals under their care and to design strategies to help ensure that the trace element requirements of the ruminant are met and that good health status can be established and maintained. This should enable them to better resist the every day challenges by pathogenic organisms responsible for many of the current infections such as mastitis. However, currently we only have enough information to be able to do this with any degree of certainty by using dietary supplementation with appropriate trace elements which is a conventional approach and does not sit well with organic philosophy.

There are indications from a very small number of studies that there may be a more “sustainable, organic” way to achieve the same objective by increasing the diversity of the pastures used as the major food source for ruminant livestock. However, much more information is needed on the levels of trace elements present in these plants, how the level varies with soil trace element content and health status of the soil and how the level varies with stage of growth through the season. Only when we have this more detailed information can we design pastures that provide for the animal’s requirements.

An additional benefit of such pastures is that they may result in a much greater diversity of wildlife.

Attention also needs to be paid to the management and nutrition of the livestock in order to reduce both physical and nutritional stress to a minimum.

Recommendations for future research.

A considerable body of research has now given a reasonable though still incomplete insight into the role of trace elements in humans and animals and their importance in maintaining good health. The existing information could be used to make a start in improving in animal health, particularly mastitis and somatic cell counts in dairy cattle, if applied in a logical and sensible way in a number of situations. There are also problems with fertility in all ruminants that may in part be improved by improving trace element nutrition, though often poor fertility is a result of energy deficit at a critical point in the cycle. Part of the problem appears to be that forages grown on some soils are deficient in particular trace minerals resulting in a deficiency in the livestock dependent on that forage (Tame 2006, Measures 2006). At present a large proportion of the grassland used for forage for both grazing and conservation for dairy cattle is ryegrass based and this appears to exacerbate any deficiency. This may perhaps be less so for beef and sheep where a larger proportion of their feed is derived from permanent pasture. However, only a relatively small proportion is ”species rich” permanent pasture containing herbs.

In the short term it is permissible to tackle trace mineral deficits by feeding supplementary minerals or boluses or in extreme cases giving injections of the appropriate mineral in an appropriate form. However, from a philosophical point of view this is farm from satisfactory and only serves to alleviate the problem, usually only on a temporary basis, once it has arisen rather than to prevent the problem arising. In order to prevent problems arising, we need to take a wider view of livestock feeding and understand much more about the accumulation of trace elements by plants and how this varies with stage of growth and growing conditions. We also need to better understand soil/plant interactions and how uptake of trace elements can be influenced by “re-balancing” soils either by supplementation directly with trace elements or by improving the biological activity of soils.

The following are some suggestions for areas to be covered by future research projects. Their aim should be to gather information that will enable us to devise forage/herb mixes that will fully provide the trace mineral levels necessary to establish and maintain a good state of health including full immune competence in ruminants.

1) A comparison of the trace element and vitamin content of a range of grasses, legumes and herbs at various growth stages. A number of different trials need to be run on a range of soil types including soils known to be deficient in particular trace minerals and/or to result in trace element deficiencies in ryegrasses.

2) The effect of “trace element rich” forages on animal health with particular reference to mastitis and SCC.

3) A study needs to be undertaken on whether the various soil management strategies practiced at present do indeed enhance the trace mineral level of forages grown.

References

Alderman, G.(1993), *Energy and Protein requirements of ruminants*, CABI Int.

ARC (1980), *Nutrient Requirements of Farm Livestock, Commonwealth Agricultural Bureaux, Farnham Royal, Slough, UK.*

Astrosi, F, Tyopponen, J, Sankari, S, Kangasniemi, R and Parantainen, J, (1986), *Int., J. Vitamin Nutr. Res.* 57:37

Astrosi, F, Rizzo, A, Kangasniemi, R, Snakari, S, Tyopponen, J, Osterman, T and Parantainen J, (1989), *J. Vet. Med.*, **36:**702

Belesky, D P, Fedders, J M, Turner, K E and Ruckle, J M, (1999), *Agron. J.,* **91:**450

Boland, M P (2003) *Adv. Dairy Technology*, **15:** 319-328

Clarke, T W, Xi, Z, Du, Z, and Hempken, R W, (1993), *J. Dairy Sci.,* **76(suppl):**462

Craven, N. & Williams, M.R. (1985), *Vet. Immunology and Immunopathology*, **10,** 71

Diplock, A T, (1981), *In: Selenium in Biology and Medicine, AVI Publishing, Hartford, CT.* p 303

Eberhart, R.J. (1986), *J. Dairy Science*, **69,** 1721

Erskin, R J, (1993), *The Veterinary Clinics of North America: Food Animal Practice*, Publ: W B Saunders, Philadelphia, PA. p 551

Van Ekeren, N, Wagenaar, J P and Jansonius, P J, (2006)

Erskine, R J, Ebehart, R J, Hutchinson, L J & Scholz, R W (1987) *J. Am.Vet. Med. Assoc.*, **190:**1417

Givens, D I, Allison, R, Cottrill, B & Blake J S, (2004), *J. Sci Fd. & Ag*, **84:**811

Goff, J P & Stabel, J R, (1990), *J. Dairy Sci.,* **73:**3195

Goodwin-Jones (2007) personal communication

Grace N D, Lee, J, Mills, R A and Death, (1997), *N. Z. J. Agric. Res., **40:**75*

Grohn, Y.T. & Rajala-Schultz, P.J. (2000), *Animal Reproduction Science,* **60-61:**605

Hamon, R J, & Torre, P M, (1994), *Proc. Natnl Mast. Counc., Orlando, Florida,* pp 54

Harrington, K.C., Thatcher, A. and Kemp, P.D. (2006), *New Zealand Plant Protection,* **59:**261

Hemingway, R.G. (1999), *Vet. Res. Comm.,* **23:**481

Johnston, L.A. & Chew, B.P. (1984), *J. Dairy Sci.,* **67:**1832

Jung, G A, Shaffer, J A, Varga, G A and Everhart, J R, (1996), *Agron. J.*, **88:**104

Kellog, D.W. (1990), *Feedstuffs,* **20:**15

McDonald, P, Edwards, A, Greenhalgh, J, (1995), *Animal Nutrition (5th ed),* Longman

Measures, M. (2006) personal communication

Meglia, G.E. (2004), *Doctoral Thesis*, Nutrition and Immune Response in Periparturient Dairy Cows, Swedish University of Agricultural Sciences, Upsala, Sweden.

Mulryan, G, & Mason, F, (1992), *Ann. Res. Vet.*, **23:**233

NRC (2001), *Nutrient Requirements of Dairy Cattle, Natl. Acad. Press, Washington DC*

Oldham, E.R., Eberhart, R.J. and Muller, L.D. (1991), *Journal of Dairy Science*, **74:**3775

Organic Livestock Research Group (2000) VEERU, Dept Agriculture, The University of Reading, Compendium of Animal Health and Welfare

Ostergaard, S & Sorensen, J.T. (1998), *Preventative Vet. Med.*, **36:**109

Pechova, A, Pavlata, L and Lokajova, E, (2006), *Acta Vet. Brno*, **75:**355

Parker & Page (1994)

Prasad, A S (1979) *Ann. Rev. of Pharm. and Toxicol.*, **19:** 393

Puls, R, (1990), *Mineral levels in animal health, Sherpa International, Clearbrook, British Columbia, Canada.*

Putnam, M.E. & Comben, N. (1987), *The Vet. Rec.*, **121:**541

Rice, D A & Kennedy, S, (1988), *Proc. Nutr. Soc.*, **47:**177

Roderick, Stephen and Hovi, Malla (2001), *Organic Livestock: Animal Health, Welfare and Husbandry Assessment of existing knowledge and production of an advisory resource compendium.* Report, Veterinary Epidemiology and Economics Research Unit (VEERU), University of Reading. *Orgprints website ref: http://orgprints.org/6663*

Rukkwamsuk, T., Kruip, T.A.M. and Wensing, T. (1999), *Vet. Quarterly*, **21:**71

Sanderson, M A, Labreveux, M, Hall, M H and Elwinger, G F, (2003), *Crop Sci.*, **43:**1797

Scaletti, R W, Trammell, D S, Smith, B A & Harmon, R J, *Int. Mast. Symp. Natl. Mast. Counc./Am. Assoc. Bovine Practitioners*, pp 29

Smith, K.L., Hogan, J.S. and Weiss, W.P. (1997), *J. Anim. Sci.*, **75:**1659

Smith, K.L. & Hogan, J.S. (2001), *proceedings of the 2nd International Symposium on mastitis and milk quality*, Vancouver, Canada, pp 1

Swenson, C K, (1998), *Ph D Dissertation NM State University, Las Cruces.*

Tame, M.J. (1995) personal observations
Tame, M.J. (2006) personal observations

Tomlinson, D J, Scocha, M T, Rapp, C J and Johnson, A B, (2002), *J. Dairy Sci.*, **85(suppl 1):**106

Terry, N, Zayed, A M, de Souza, M P and Tarun, A S, (2000), *Annual Review of Plant Physiology and Plant Biology*, **51:**401

Underwood, E.J. and Suttle, N.F. (1999) in The Mineral Nutrition of Livestock, 3rd edition, Publ. CABI International, Wallingford, Oxford, OX10 8DE.

Weller, R F, & Bowling, P J, (2001) In: *Organic meat and milk from ruminants, Proc. Int. Conf. Org. Meat & Milk*, Athens

Weiss, W P, (2002), *Proc. Natl. Mast. Counc. Ann. Meeting*, pp 37