Se Toxicity found in QLD Northern Territory

Se Def. — WA VIC NSW TAS.

SELENIUM IN ANIMAL PRODUCTION IN AUSTRALIA

D.W. PETER* and N.D. COSTA**

Summary

Selenium in animal production in Australia has followed a similar path to that in most regions of the world, being first considered as a toxin and then, once identified as an essential element, as the cause of significant pathological disorders and the cause of sub-clinical losses in production. This paper briefly describes the history of selenium toxicity and deficiency in Australia's livestock industries, and provides an outline of the some of the most recent aspects of selenium research related to animal production.

I. INTRODUCTION

Some 140 years elapsed following the discovery of selenium as an element before studies by Schwartz and Foltz (1957) in the USA resulted in its identification as essential nutrient for the rat. Following this discovery it was rapidly shown to be also essential for poultry (Patterson et al. 1957) and ruminants (Muth et al. 1958), although demonstration of its essentiality for humans was delayed for approximately a further two decades.

Until selenium was shown to be an essential nutrient, the only biological role for this element was as a highly toxic substance, a feature first identified by Japha in 1842 (Japha 1842) and given wide attention in the USA in the 1930's. During this latter period it was first shown that selenium, in wheat grown in South Dakota, was toxic to rats (Robinson 1933) and that high levels of selenium, present in grains and grasses grown on specific soil types, were associated with the well recognised disorders of livestock known as "blind staggers" and "alkali disease" (Franke 1934). At the same time, Beath and colleagues (1934) showed that selenium was also the toxic agent in certain species of range plants, in species of *Astragalus* in particular, growing in seleniferous soils in the mid west of the USA, and responsible for the natural occurrence of "alkali disease". Since that time it has been shown that selenium may be somewhat of an artefact in the "blind staggers" syndrome.

In hindsight, the toxic effects of excess selenium were probably the major factors which delayed the consideration of its essentiality to animals. Furthermore, even after its essential role became known, the toxicity of excess selenium continued to affect attitudes to its use in animal production and possible functions in biological systems; concern over toxicity still persists to some extent today.

---

*CSIRO Division of Animal Production, Private Bag, PO, Wembley, Western Australia 6014
**School of Veterinary Studies, Murdoch University, Murdoch, Western Australia 6150

II. THE HISTORY OF SELENIUM IN ANIMAL PRODUCTION IN AUSTRALIA

(a) Toxicity

As in other parts of the world the first recognised and documented role for selenium in animal production in Australia was as a naturally occurring plant toxin. Identification of natural selenosis in livestock occurred much later in Australia than in the USA with the first published report of toxicity taking place one year after the discovery that selenium was an essential element. Knott et al. (1958) reported that horses which grazed the shrub, *Morinda reticulata,* in an area of Cape York peninsular in Queensland developed laminitis and the associated classical clinical symptoms of "alkali disease". Subsequent investigations showed that this plant is a true selenium accumulator and that the high levels of selenium in its tissues do not necessarily arise due to excessively high levels of selenium in the soil. In retrospect it appears that selenosis had occurred in the region since first settlement.

Selenosis has also been observed in sheep and horses in northwest Queensland in a region between Hughenden and Julia Creek. In this region a number of native plant and grasses growing on soils derived from or associated with Toolebuc Limestones have been found to accumulate high and potentially toxic levels of selenium. On one property, where an outbreak of selenosis was observed, levels in excess of 4000ppm were recorded in *Neptunia amplexicaulis*, a shrub now considered to be an indicator plant (Knott and McCray 1959). Petersen and Butler (1967) showed that the majority of selenium in this plant is present in two water soluble amino acids, with one third as selenocystathionine. Mild selenosis has commonly been observed in horses and although confirmed only once in sheep it is suspected that it may be one of the causes of a local disorder termed "humpy back".

There have been no reports of endemic selenosis in states other than Queensland, although acute and chronic intoxication arising from incorrect therapeutic dosing, to correct or prevent selenium deficiency in sheep and calves, has been widely reported. The most numerous cases occurred in Western Australia in the years immediately following the recognition of selenium deficiency in grazing sheep (Gabbedy 1970).

Apart from a considerable difference in the toxicity of inorganic and organic forms of selenium there is no clear consensus on the minimum levels of inorganic selenium that are toxic to grazing livestock. Toxicity appears to depend on a wide variety of factors including species, age, physiological state, liveweight, nutrition and diet, as well as the form of selenium and route of administration. There is, nevertheless, a wide margin of tolerance between beneficial and toxic levels of selenium, as demonstrated by the studies of Pierce and Jones (1968) with sheep. Levels of sodium selenite in excess of 200 μg/kg liveweight per day were needed to reduce feed intake and wool growth in adult sheep, with sheep of 50kg tolerating administration of 30mg Se/day for an extended period. Such results clearly show that, apart from the possibility, albeit unlikely, of unacceptably high levels of selenium in edible products, concerns over possible toxicity from the use of commercial, selenised fertiliser and selenium supplemented feedstuffs are unfounded, if currently recommended rates of use and NH&MRC guidelines respectively are adhered to.

(b) Deficiency

The subject of selenium deficiency has been extensively reviewed, with a comprehensive paper by Judson et al. (1987) in relation to ruminants in particular. This paper therefore only presents a summary of major points and the most recent information.

Prior to 1957 there was the odd clinical description of a "disease" of unknown aetiology affecting livestock in Australia and producing muscle necrosis - "white muscle" or "stiff lamb disease" - and at times death. During the early to mid 1950's, following the discovery that vitamin E could prevent liver necrosis in chickens (Scharwz et al. 1957; Patterson et al. 1957) and muscle necrosis in lambs (Muth et al. 1958), it was thought that inadequate vitamin E may be responsible for the occasional outbreak of this "disease". A number of trials were conducted in New Zealand using Vitamin E supplementation. These had mixed and inconclusive results. A similar trial with vitamin E in NSW failed to find any beneficial effect of the vitamin in preventing white muscle disease in lambs.

The discovery that selenium was an essential element rapidly resulted in the recognition in Australia of the natural occurrence of selenium deficiency in livestock. Somewhat surprisingly, the observed incidence of "white muscle disease" and selenium responsive ill-thrift in young growing sheep appeared to steadily increase in the following decade. Attempts to determine whether this steady increase was due to the corresponding increases in pasture improvement, the use of superphosphate fertiliser and in stocking rates, or other factors, were by and large unsuccessful. All these factors were probably involved. For example, it is known that the concentration of selenium in sub-clover, which was being widely introduced into pastures over this period, is generally lower than that in grass when both are grown together in a mixed sward (Whelan 1989), that their are affects of pasture growth rate on the concentration of selenium and that selenium status and responses to selenium supplementation of grazing sheep are influenced by stocking rate (Langlands et al. 1982). In addition, the fact that the correction or prevention of various other causes of losses in productivity and signs of ill-health, e.g., GIT parasites, other mineral deficiencies, poor summer nutrition, was occurring over this same period would have made signs of selenium deficiency more conspicuous.

Although the occurrence of selenium deficiency has now been recognised in Australia for over 30 years the discovery of sub-clinical deficiencies in some regions is still continuing. During the mid 1980's a survey carried out by Judson and colleagues of blood selenium levels in cattle and sheep in the southeast of South Australia revealed that significant numbers of animals were at risk of having reduced production due to inadequate selenium (Judson et al. 1987). Even more recently, studies by Norton et al. (1990) in south west Queensland and Holmes (1992) in south eastern Victoria have shown responses in wool growth in young sheep following multi-element, including selenium, supplementation where none have previously been recorded. In the latter case, the area was previously considered to be potentially deficient in selenium on the basis of selenium concentrations in pastures.

Although one of the reasons why sub-clinical deficiencies are not detected obviously rests with the lack of visible or clinical signs of deficiency, several further factors play an even more important part. It is commonly claimed that the only way to guarantee that selenium (or other minerals) are deficient is to supplement and gain a response. The results of Masters and Peter (1990) in WA and, to a lesser extent, Holmes (1992) in south-east Victoria, however, highlight the problem of using this approach. In both these studies supplementation of weaner sheep with selenium alone failed to produce a beneficial response in production. However when cobalt, and in Victoria, copper, as well as selenium were administered together, there were significant responses in wool growth. Consequently, the diagnosis of deficiency by measuring the response to supplementation may be concealed if there is also a second (or third) limiting element.

The second major problem that exists in diagnosing sub-clinical deficiencies is the difficulty in using measurements of selenium concentrations or glutathione peroxidase activities in blood or liver. This problem is not unique to Australia but exist world wide, with differences in the minimum blood and tissue concentrations between different regions at which animals are

positively respond in some way to selenium supplementation. As a result, tables which provide levels of selenium for diagnosis generally include a lower and upper limit, below which responses are assured, an upper, or will not occur repesctively, as well as a "maybe" range. For example, the extensive studies of Langlands and co-workers (1991a) in the New England region of NSW indicate that whole blood values of 0.02ppm or less are needed before responses to selenium supplementation will occur and a "maybe" range is not clearly evident. On the other hand, Dove et al. (1986), in a study at the Ginninderra Experiment Station near Canberra, reported increased weaning weights and fleece weights in Merino lambs with GSH.Px activities of 46 U/g Hb in the untreated group. Similar responses occurred in some regions of WA when whole blood levels fall below only 0.05 ppm (Judson et al. 1987), a point recently demonstrated yet again by the studies of Whelan et al. (1993a). Using the relationship between liveweight response and blood selenium levels in sheep, they showed that liveweight responded to supplementation up to whole blood and plasma concentrations of 0.06 ppm and 0.03 ppm respectively. The equivalent value for muscle were 0.035 and 0.14 ppm on a wet weight and dry weight basis respectively (B. Whelan, pers. com.).

The value for muscle is similar to the value of 0.1 ppm reported by Peter et al. (1987), who also suggested that the difficulties in using liver and blood concentrations for diagnosis arise because of their poor correlation with muscle concentrations. Concentrations in plasma and liver were found to be closely related to dietary selenium concentration, irrespective of selenium intake, whereas muscle concentrations were more closely aligned with selenium intake.

Apart from the effects of selenium intake in the diet and dietary concentration per se there is also the effect of the form of selenium ingested. It is now widely recognised that the availability of organic forms of selenium are generally much higher than those of inorganic. This applies particularly to ruminants, where metabolism of selenium in the rumen renders a significant proportion of ingested selenium unavailable for absorption and the conversion of inorganic selenium to these unavailable forms is greater than for organic forms (Peter et al. 1986; Whanger et al. 1968; P.D. Whanger and D.W. Peter, unpubl. data). It has been suggested that the higher uptake of absorbed forms of organic selenium by muscle tissue and its incorporation into protein is due to non-specific incorporation of seleno-amino acids into protein in place of sulphur amino-acids. Studies with mice have been unable to identify any differences in protein profiles from animals given labelled seleno-methionine or methionine (Burk 1991). However, this does not negate the possibility that there are differences in biological activity between proteins incorporating seleno-methionine as opposed to methionine. For example, glutathionine peroxidase was for a long time considered to be the only active seleno-enzyme. Since that time, selenoprotein P, which is thought to have a role in the transport of selenium between the liver and extrahepatic tissues, has been reported in plasma from rats (Motsenbocker and Tappel 1982) and sheep (Davidson and McMurray 1987). In addition to GSH.Px and selenoprotein P, Behne et al. (1990) have identified at least ten other ^{75}Se-labelled proteins in liver homogenates from rats. Several further seleno-enzymes exist in microbial systems (Stadtman 1990). Whether these enzymes, or others, may be important for optimum microbial function in the rumen, and therefore essential to ruminants, has however, to the best of our knowledge, never been investigated.

Apart from the seleno-proteins in tissues, there is good evidence showing significant beneficial effects of organic selenium compounds, some synthetic, as therapeutic agents in the prevention or reduction of selected cancers, as anti-inflammatory agents and as anti-oxidants per se (not as GSH.Px) in animals (Milner, 1985; Parnham and Graf 1991). The possibility, therefore, cannot be dismissed that some natural organo-selenium compounds in plants eaten by

grazing animals may have similar functions and that differences in their occurrence and reactivity between regions may influence the critical requirements for selenium.

It has been suggested that the difference between areas and between seasons in the occurrence of selenium responsive conditions is due to some factor(s) other than selenium per se (Gabbedy 1977; Hunter et al 1982). Factors such as stocking rate, pasture composition, rainfall pattern and plant growth rate, which influence animal intake and growth rate, as well as the animal's physiological state and genotype, may also be indirectly involved. It is now clear that the intake of vitamin E can also have an effect by altering the requirement for selenium. In regions with annual pastures and extended periods when only dry pasture is available for ingestion, vitamin E intake and hence animal status declines, creating a need for higher levels of selenium as GSH.Px. If vitamin E intakes remain low for long enough vitamin E status falls to a level which no amount of selenium as GSH.Px can compensate for and frank vitamin E deficiency is seen (Steele et al. 1981; McDonald and Caple 1983). Unfortunately, the clinical symptoms of vitamin E deficiency are identical to those of selenium and hence vitamin E deficiency may persist if it is assumed that the symptoms are due to selenium deficiency and animals treated accordingly. Work is still proceeding on determining whether sub-clinical vitamin E deficiency exists.

Presumably, differences between locations, or between seasons, in the intake of dietary compounds likely to induce the formation of free radicals or affect the intake or availability of iodine and the synthesis and availability of thyroid hormones will also alter selenium requirements.

II. RECENT ASPECTS OF SELENIUM IN ANIMAL PRODUCTION IN AUSTRALIA

(a) General

As noted above, the identification of areas where inadequate selenium may affect the productivity of grazing livestock continues, either through measurement of the selenium concentration in blood collected from grazing animals or through the testing of the effects of selenium supplementation on production. It is anticipated that as greater emphasis is placed on the quality of products, e.g., staple strength in wool, and on "sustainable" production, the effects of marginal deficiencies will assume greater importance. Consequently, attempts will be made to identify those areas with the most marginal risk of losses in productivity due to inadequate selenium. The tendency to treat animals on an insurance rather than an assured response basis will therefore become even more prevalent, provided treatment costs remain low.

(b) Sheep

Recent studies have shown that the variability in the long term effectiveness of intraruminal selenium pellets for correcting selenium deficiency in sheep can be overcome by also increasing the selenium content from 5 to 10% (Langlands et al. 1993). The use of selenium fertiliser to increase the selenium content of pastures and thereby increase the selenium status of animals (sheep) grazing those pastures has also recently been brought into use in Australia as an alternative to using selenium pellets, drenches or injections (Halpin et al. 1985; Whelan et al. 1993 a,b). Studies in WA, with a slow release form of selenium fertiliser containing barium selenate, have shown that a single application was effective in maintaining the selenium status of grazing sheep for a minimum of three years (Whelan 1993b). A selenium fertiliser, consisting of a combination of a rapid release and a slow release form, is now being

marketed by Cooper Pitman-Moore Ltd under the trade name Agsel®. It is understood that other companies in Australia are also marketing selenium containing fertilisers.

Over the past decade there has been considerable interest in the occurrence of non-selenium responsive, nutritional myopathies related to vitamin E deficiency. Unfortunately, attempts at studying the problem in the field are difficult. Small amounts of rain during the summer-autumn period and the emergence of small quantities of green pasture immediately improves vitamin E intake and status. Results from year one of a field study being carried out in a low selenium region in WA have shown that treatment with either selenium or vitamin E or both can influence clean wool growth or staple strength (D.W. Peter, unpublished data). If these results can be confirmed it would appear that the occurrence of sub-clinical vitamin E deficiency is unlikely, provided selenium status is adequate. Consequently, the only effect of vitamin E on production may be during frank clinical deficiency and this can occur in both selenium adequate as well as selenium deficient regions.

In addition to the vitamin E responsive myopathies that occur when selenium is adequate, there is an apparent further "non-selenium responsive" myopathy associated with sheep grazing lupin stubbles infected with *Phomopsis leptostromiformis*. The phomopsin toxins associated with this fungus can result in lupinosis associated myopathy (LAM), which fails to respond to treatment with either inorganic selenium or vitamin E or both (Allen et al. 1979; Allen et al. 1992). More recent studies still suggest a role for selenium and vitamin E in LAM (S.A. Beetson, N.D. Costa, D.W. Peter and J.G. Allen, unpublished data).

The variability in responses to selenium supplementation continues to makes it difficult to assess the economic benefits of preventing or correcting selenium deficiency in the Australian sheep flock. Unfortunately, this variability is not only confined to variation between regions or between farms in the same region, etc, but also in the nature of the response. For example, selenium supplementation of sheep in the New England region of N.S.W. has been found to improve ewe fertility (Piper et al. 1980; Wilkins and Kilgour 1982a), the number of lambs weaned per ewe mated, the percentage of lambs weaned and wool production (Wilkins and Kilgour 1982b; Langlands et al. 1991a,b,c). In contrast, responses in W.A. have been confined to liveweight and wool production (Gabbedy et al. 1977; Judson et al. 1987). Unfortunately, increases in wool production in New England have also been found to be accompanied by an increase in fibre diameter (Langlands et al. 1991a), whereas fibre diameter can be unaffected in W.A.(Whelan et al. 1993b; D.W. Peter, unpublished data). Consequently, the benefits from selenium supplementation in the two regions can vary according to the premium paid for fibre diameter. Understanding the causes of variations in response and improving diagnostic procedures therefore still remain as priorities for sheep production in Australia.

(c) Cattle

Selenium responsive disorders are less well defined in cattle as compared to sheep. Nutritional myopathy has been reported in weaned calves in Victoria (Allen and Friend 1978), but most of the data on selenium status of cattle stem from the analysis of blood samples collected for the National Brucellosis Eradication Scheme in NSW (Langlands et al. 1981) and SA (Judson et al. 1987; McFarlane and Judson; 1990). Although there are large numbers of cattle deemed to be at risk of selenium deficiency by the criteria of whole blood Se of 20ng/ml or less (Langlands et al. 1981), the response to selenium supplementation has been patchy at best. Significant responses in live weight were reported only in herds which showed illthrift and diarrhoea (Langlands et al. 1989), under which conditions vitamin E absorption may be diminished(Rice and Kennedy 1988).. Reference curves showing the relationship between

response to supplementation and indices of mineral status, as proposed by Clark et al. (1985), were of limited use in the Langlands study. The response to selenium supplementation of calves has been relatively unsuccessful in Victoria with only one response in 30 trials during the period 1970 - 1984 (Sully et al. 1982) but more recently another positive response has been found in the East Gippsland area (D. Phillips pers. commun.). Nevertheless the reasons for the poor and variable response to selenium supplementation in beef cattle, as with sheep, remain to be elucidated.

The circumstances of selenium responsive disorders in dairy cattle in Australia are equally unclear. In studies outside of Australia, low blood selenium in dairy cows has been associated with improvements in milk production in the North Island of New Zealand (Fraser et al. 1987; Tasker et al. 1987) and more commonly, reproductive disorders such as retained foetal membranes (Segerson et al. 1981) and mastitis (Ndiweni et al. 1991). Supplementation of dairy cows with selenium pellets in NSW has resulted in a decreased risk of subclinical mastitis (Ryan 1985), increased growth rates in supplemented growing heifers (Lean, I.J. pers. comm.), and in increased conception rates (McClure et al. 1986). The herds in the study by McClure et al. did not have a high incidence of retained placentae and in fact would not qualify as selenium deficient by the criteria of Langlands et al. (1981) because their blood GSH.Px values were $\geq$ 39 U/g Hb. However in Victoria, retained foetal membranes in dairy cows were not associated with selenium deficiency, nor was there any production responses to selenium supplementation of dairy cows in South Gippsland (Caple et al. 1982). Given the variability in responses seen in dairy cattle, Grace (1988) has suggested that they may be less sensitive to low Se intakes than sheep, notwithstanding that selenium absorption in dairy cows is very low, of the order of 10 - 16% (Koenig et al. 1991; Hood and Costa 1992). It may be premature to draw such a conclusion until other factors such as polyunsaturated fatty acid intake, fatty acid and nucleic acid peroxidation, and the intake of antioxidants such ß-carotene and vitamins E and C, in addition to the selenium status are taken into account when considering problems of reproductive and production performance in dairy cows.

(d) Other species

Conditions attributed to dietary deficiencies of selenium in pigs and poultry are infrequent in Australia. Edwards et al. (1975), in a commercial piggery near Sydney, reported a significant increase in the conception rate of gilts to first service in response to injections of sodium selenite. This effect was observed at a dietary selenium concentration of 0.1 ppm. Moir and Masters (1979) reported on a number of outbreaks of nutritional myopathy, hepatosis dietetica and mulberry heart disease in WA where meatmeal was replaced in the ration by lupinseed meal, and barley of low selenium content. Importantly, while hepatic selenium concentrations were significantly lower in cases of nutritional myopathy and hepatosis dietetica, there were no differences in cases of mulberry heart disease. From this finding, they suggested that mulberry heart disease was not a selenium responsive disorder. Rice and Kennedy (1989) confirmed that mulberry heart disease was associated with impairment of α-tocopherol metabolism with no related changes in dietary selenium and PUFA content, as well as no changes in tissue selenium and GSH.Px. However, Nielsen et al. (1989) found mulberry heart disease still persists among young pigs in Denmark despite the addition of abundant supplies of selenium and vitamin E to feedstuffs. Once again, factors such as genotype and realisation of genetic potential through interaction of diet and environment need to be considered.

Selenium responsive disorders in poultry are characterised by exudative diathesis, nutritional myopathy, nutritional pancreatic atrophy, and depressed appetite (Combs and Combs 1984). Bains et al. (1975) reported these conditions in a commercial poultry organisation in Queensland where broiler breeder birds presented with exudative diathesis, nutritional myopathy, and nutritional pancreatic atrophy from 24 - 63 weeks of age and their

progeny showed exudative diathesis at seven days and nutrition myopathy at six weeks. Sinclair et al. (1984) reported pancreatic degeneration associated with reduced GSH.Px activity and occasionally lower plasma vitamin E in broilers with runting and stunting syndrome.

IV. THE FUTURE

In the extensive grazing industries (sheep and cattle) treatment with effective, long-acting selenium pellets to protect against the occurrence of losses in production from sub-clinical deficiencies, is likely to increase. There may also be much wider use of selenium fertiliser although, despite the apparent safety of this procedure, the fear of toxicity may, particularly from an environmental viewpoint, cause constraints. Provided selenium is routinely included as part of the rations for pigs and poultry, which currently appears to be the case, no serious problems or changes appear likely in future in these industries. New challenges will arise in potentially new livestock industries such as emu and kangaroo farming, where selenium and vitamin E requirements appear to be greater than for the currently domesticated species.

From a scientific stand point, there is undoubtedly still a need to understand why selenium requirements are variable, why supplementation can increase wool growth in some situations without appearing to alter liveweight or, likewise, to increase fibre diameter of wool in some circumstances and not in others, etc. It is possible that some of these differences may be related to the role of selenium in thyroxine metabolism and its association with the regulation of metabolic rate or interactions with other hormones regulating growth, etc. However, despite the apparent non-specificity of incorporation of selenium and particularly seleno-amino acids, such as seleno-methionine, into many tissue proteins, the likelihood of an active role for some of these proteins appears high.

REFERENCES

ALLEN, J.D. and FRIEND, S.C.E. (1978). Aust. vet. J. 54: 547.
ALLEN, J.G., WOOD, P.MCR., CROKER, K.P. and HAMBLIN, J. (1979). J. Agric. W.A. 20: 10.
ALLEN, J.G., STEELE, P., MASTERS, H.G. and D'ANTUONO, M.F. (1985). Aust. vet. J. 63: 8.
ALLEN, J.G., STEELE, P., MASTERS, H.G. and LAMBE, W.J. (1992). Aust. vet. J. 69: 75.
BAINS, B.S., MACKENZIE, M.A. and McKENZIE, R.A. (1975). Aust. vet. J. 51: 140.
BEATH, O.A., DRAIZZE, J.H., EPPSON, H.F., GILBERT, C.S. and McCREARY, D.G. (1934). J. Amer. Pharm. Assoc. Sci. Ed. 23: 94.
BEHNE, D., SCHEID, S., KYRIAKOPOULOS, A. and HILMERT, H. (1990). Biochim. Biophys. Acta. 1033: 219.
BURK, R.F. (1991). Faseb. J. 5: 2274.
CAPLE, I.W., ANDREWARTHA, K.A., EDWARDS, S.J.A. and HALPIN, C.G. (1980). Aust. vet. J. 56: 160.
CAPLE, I.W., McCLORY, J.W. and HALPIN, C.G. (1982). In "Trace Element Review papers, 1982". Agric. Service Library, Dept. of Agric. Vic.
CLARK, R.G., WRIGHT, D.F. and MILLAR, K.R. (1985). N.Z. Vet. J. 33: 1.
COMBS, G.J. and COMBS, S.B. (1984). Ann. Rev. Nutr. 4: 257.
DAVIDSON, W.B. and McMURRAY, C.H. (1987). J. Inorg. Biochem. 30: 1.

DONALD, G.E., LANGLANDS, J.P., BOWLES, J.E., SMITH, A.J. and BURK, G.L. (1993). Anim. Feed Sci. Technol. (Submitted for publication).
EDWARDS, M.J. . HARTLEY, W.J. and HANSEN, E.A. (1977). Aust. vet. J. 58: 553.
FRANKIE, K.W. (1934). J. Nutr. 8: 597.
FRASER, A.J., RYAN, T.J. CLARK, R.G. and SPROULE, R. (1987). Proc. 4th Asian-Australasian Assoc. Anim. Prod. p 427.
GABBEDY, B.J. (1970). Aust vet. J. 46, 223.
GABBEDY, B.J., MASTERS, H. and BODDINGTON, E.B. (1977). Aust. vet. J. 53: 482.
GRACE, N.D. (1988). Proc. Aust. Soc. Anim. Prod. 17: 42.
HALPIN, C.G., McDONALD, J., HANARAHAN, P. and CAPLE, I.W. (1985). In "TEMA-5", p746. Eds C.F. Mills, I. Bremner and J.K. Chesters. C.A.B., London.
HOLMES, J.H.G. (1992). Aust. vet. J. 69: 292.
HOOD, G.M. and COSTA, N.D. (1992). Proc Nutr. Soc. Aust. 17: 147.
HUDSON, D.R., HUNTER, R.A. and PETER, D.W. (1981). Aust. J. Agric. Res. 32: 935.
HUNTER, R.A., PETER, D.W., QUINN, M.P. and SIEBERT, B.D. (1982). Aust. J. Agric. Res. 33: 637.
JAPHA, A. (1842). Dissertation Halle 1842. Cited by Moxon, A.L. & Rhian, M. (1943). Physiol Rev. 23, 305.
JUDSON, G., LANGLANDS, J.P., CAPLE, I.W. and PETER, D.W. (1987). Chpt. 18. in "Temperate Pastures: Their Production, Utilisation and Management". Eds J.L. Wheeler, C.J. Pearson and G.E. Robards. CSIRO: Melbourne.
KNOTT, S.G., McCRAY, C.W.R. and HALL, W.T.K. (1958). Qld. J. Agric. Sci. 15: 43.
KNOTT, S.G. and McCRAY, C.W.R. (1959). Aust. vet. J. 35: 161.
KOENIG, K.M., BUCKLEY, W.T. and SHELFORD, J.A. (1991). Can. J. Anim. Sci. 71: 167.
LANGLANDS, J.P., BOWLES, J.E., DONALD, G.E. and SMITH, A.J. (1982). Aust. J. Agric. Res. 33: 313.
LANGLANDS, J.P., BOWLES, J.E., DONALD, G.E. and SMITH, A.J. (1986). Aust. J. Agric. Res. 37: 201.
LANGLANDS, J.P., DONALD, G.E., BOWLES, J.E., and SMITH, A.J. (1989). Aust. J. Agric. Res. 40: 1075.
LANGLANDS, J.P., BOWLES, J.E., DONALD, G.E. and SMITH, A.J. (1990). Anim. Feed Sci.Technol. 28: 15.
LANGLANDS, J.P., DONALD, G.E., BOWLES, J.E. and SMITH, A.J. (1992a). Aust. J. exp. Agric. 31, 25.
LANGLANDS, J.P., DONALD, G.E., BOWLES, J.E. and SMITH, A.J. (1992b). Aust. J. exp. Agric. 31, 33.
LANGLANDS, J.P., DONALD, G.E., BOWLES, J.E. and SMITH, A.J. (1992c). Aust. J. exp. Agric. 31: 37.
McCLURE, T.J., EAMENS, G.J. and HEALY, P.J. (1986). Aust. vet. J. 63: 144.
McDONALD, J.W. and CAPLE, I.W. (1983). Proc. No. 67, Sheep Production and Preventative Medicine, p 227. Postgrad. Cmtee Vet. Sci., Univ. Sydney, Sydney.
McFARLANE, J.D. and JUDSON, G.J. (1990). Proc. Aust. Soc. Anim. Prod. 18: 524.
MILNER, J.A. (1985). In "Xenobiotic metabolism: Nutritional effects. ACS Symp. series 277, p267. Eds J.W. Finley and D.E. Schwass.
MOIR, D.C. and MASTERS, H.G. (1979). Aust. vet. J. 55: 360.
MOTSENBOCKER, M.A. and TAPPEL, A.L. (1982). Biochim. Biophys. Acta 719: 147.
MUTH, P.H., OLDFIELD, J.E., REMMERT, L.F. & SCHUBERT, J.R. (1958). Science 28: 1090.
NDIWENI, N., FIELD, T.R., WILLIAMS, M.R., BOOTH, J.M. and FINCH, J.M. (1991). Vet. Rec. 129: 86.
NIELSEN, T.K., WOLSTRUP, C., SCHIRMER, A.L. and JENSEN, P.T. (1989). Vet. Rec. 124: 535.

NORTON, B.W., HALES, J.W. and STOCKWELL, T.G.H. (1990). Aust. J. exp. Agric. 30: 155.
PARNHAM, M.J. and GRAF, E. (1991). Prog. Drug Res. 36: 9.
PATTERSON, E.L., MILSTREY, R. and STOKSTAD, E.L.R. (1957). Proc. Soc. Exp. Bio. Med. 95: 617.
PEET, P.L., COPLAND, M., DICKSON, J., MASTERS, H.G. and JELENIK, P. (1983). Aust. vet. J. 60: 311.
PETER, D.W., BUSCALL, D.J. and YOUNG, P. (1986). Proc. Nutr. Soc. Aust. 11: 180.
PETER, D.W., BUSCALL, D.J. and YOUNG, P. (1985). In "TEMA-5", p 487. Eds. C.F. Mills, I. Bremner & J.K. Chesters. C.A.B., London
PETER, D.W, BUSCALL, D.J. AND YOUNG, P. (1987). In "TEMA-6", P 15. Eds L.Hurley, C.L. Keen, B, Lonnerdal & R.B. Rucker. Plenum Press, New York & London.
PETERSEN, P.J. and BUTLER, G.W.(1967). Nature (Lond.) 213: 599.
PIERCE, A.W. and JONES, G.B. (1968). Aust.J. Exp. Agric. Anim. Husb. 8: 277.
PIPER, L.R., BINDON, B.M., WILKINS, J.F., COX, R.J., CURTIS, Y.M. & CHEER, M.A. (1980). Proc. Aust. Soc. Anim. Prod. 13: 241.
RICE, D.A. and KENNEDY, S. (1988). Br. Vet. J. 144: 482.
RICE, D.A. and KENNEDY, S. (1989). Am.J. Vet. Res. 50: 2101.
ROBINSON, W.O.(1933). J. Assoc. Offic. Agr. Chem. 16: 423.
RYAN, D.P. (1985). NSW Dept. Agriculture Meeting, November, p 9.1.
SCHWARZ, K. and FOLTZ, C.M. (1957). J. Am. Chem. Soc. 79: 3292.
SCHWARZ, K., BIERI, J.G., BRIGGS, G.M. and SCOTT, M.L. (1957). Proc. Soc . Exp. Biol. Med. 95: 621.
SEGERSON, E.C., REVIERE, G.J. DALTON, H.C. and WHITACRE, M.D. (1981). J. Dairy Sci. 64: 1833.
SINCLAIR, A.J., EMBURY, D.H., SMART, I.J. BARR, D.A., REECE, R.L. , HOOPER, P.T. and GOULD, J.A. (1984). Vet. Rec. 115: 485.
STADTMAN, T.C. (1990). Ann. Rev. Biochem. 59: 111.
STEELE, P., MCKENZIE, D.P., SKIRROW, S., PEET, R.L. and DONCON, G. (1981). In "TEMA-4", p210. EDS. J.McC. Howell, J.M. Gawthorne and C.L. White. Aust. Academy Science, Canberra.
SULLY, R.J., ALLEN, J.D. and CONLEY, D.N. (1982). In "Trace Element Review papers, 1982". Agric. Service Library, Dept. of Agric. Vic.
TASKER, J.B., BEWICK, T.D., CLARK, R.G. and FRASER, A.J. (1987). Proc. 4th Asian-Australasian Assoc. Anim. Prod. p 428.
WHANGER, P.D, WESWIG, P.H. AND MUTH, O.H.(1968). Fed. Proc. 27: 418.
WHELAN, B.R. (1989). Aust. J.exp. Agric. 29: 517
WHELAN, B.R., PETER, D.W. and BARROW, N.J.(1993A). Aust. J. Agric. Res. (submitted for publication).
WHELAN, B.R., BARROW, N.J. and PETER, D.W. (1993B). Aust. J. Agric. Res. (Submitted for publication).
WILKINS, J.F. and KILGOUR, R.J. (1982). Aust. J. Exp. Agric. Anim. Husb. 22: 18.
WILKINS, J.F., KILGOUR, R.J., GLEESON, A.C., COX, R.J., GEDDES, S.J. and SIMPSON, I.H. (1982). Aust. J. Exp. Agric. Anim. Husb. 22: 24.