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TRACE ELEMENTS IN ANIMAL NUTRITION

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Summary

Our knowledge of the trace element requirements of animals has increased substantially over the past decade. Five new elements have been added to the list of nutrients essential for normal functioning of animals, and many discoveries have been made concerning the biochemical role of trace elements. Due to advances in methodology, clinical symptoms of trace element deficiencies are readily diagnosed and such outbreaks have virtually disappeared from flocks and herds of animals in developed countries. The challenge of the future is to detect subclinical deficiencies and to accurately assess requirements.

I. INTRODUCTION

The past decade has seen the discovery of five, possibly six, essential trace elements (table 1), and much progress has been made in the understanding of the biochemical functions and metabolic consequences of deficiences of trace elements in general. Discoveries such as selenium in glutathione peroxidase (GSHpx) (Rotruck et al. 1973), copper in lysyl oxidase (Siegel et al. 1970) (Harris et al. 1974) and zinc in mammalian nucleic acid polymerases and ribonuclease (see review by Kirchgessner et al. 1976b) have contributed greatly to the knowledge of metabolic regulation in cells by providing a rational biochemical explanation for the clinical pathologies of trace element deficiencies.

TABLE 1. Essential trace elements for animals.

Element	Dietary requirement[1] (mg/kg)	Initial observations of deficiency symptoms	Year of discovery
I	0.1-0.8	Human goitre related to plant I levels	1852
Fe	25-100	Respiratory processes affected	1857
Cu	4-10	Poor growth and anaemia in milk-fed rats	1928
Mn	10-80	Poor growth and infertility in milk-fed rats	1931
Zn	10-50	Poor growth in milk-fed rats	1934
Co	<0.1	Enzootic marasmas in sheep and cattle	1935
Mo	<1.0	Poor growth in chickens	1956
Se	<0.1	Liver necrosis in rats fed diets containing yeast	1957
Cr	0.1	Glucose intolerance in rats	1959
Sn	1-2	Poor growth in rats fed purified diets	1970
V	0.1	Abnormal feathering in chicks and poor growth in rats	1971
Ni	0.05	Abnormal feathering and bone development in chickens	1971
F	2	Poor growth in rats. Recent data disputes this	1972
Si	500	Poor growth and bone development in rats and chicks	1972
As	<0.05	Poor growth in rats fed purified diets	1975

1 Major laboratory and agriculturally important animals.

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The past ten years have also seen the virtual disappearance of clinical trace element deficiencies in animals in developed countries, due mainly to the adoption of preventive practices such as the routine treatment of animals with trace element bullets or drenches, or addition of trace elements to feedstuffs and fertilizers. One result of this and other changes in agricultural practices has been the growing awareness of the incidence of subclinical deficiency states due to latent situations of suboptimal supply or availability.

Until recently (see section III), only four trace elements had been shown to be of economic importance for grazing animals (i.e., I, Co, Cu and Se), with zinc, iron and manganese also important for pig and poultry production. Responses to all these trace elements have been recorded in Australia, and as animal production intensifies in the tropics of Australia, new areas will undoubtably be discovered to be deficient.

This review will attempt to summarize only the more recent developments in our understanding of trace element nutrition of animals. For a more extensive discussion, readers are referred to Hoekstra et al. (1974), Nicholas and Egan (1975) and Underwood (1977).

II. ASSESSMENT OF REQUIREMENTS

Problems encountered in intensive animal production have highlighted the need for a critical appraisal of the methods commonly used to determine trace element requirements of animals. Traditional approaches include the feeding of selected diets containing adequate levels of all known nutrients, and varying the level of the trace element in question over the range from "deficient" to above adequate. The level of the element at which responses no longer occur is generally taken as the minimum requirement, with allowances being made for age, sex and physiological state (e.g. pregnancy and lactation). This data is then used in formulating dietary allowance tables. Such experiments are generally conducted under animal house conditions using semi-purified diets, and a specific disease or other responsive syndrome ascribed to the deficient trace element is used as an indicator of deficiencies in the field. This technique is limited by the fact that the values obtained are wholly dependent upon the particular animals, the diets and the criteria of adequacy. Furthermore, uncomplicated clinical symptoms observed under such experimental conditions are rarely seen in the field.

A more recent approach to determining requirements is derived from techniques used to estimate protein and energy requirements. It offers the major advantage of specifying net requirement for any production function independent of the efficiency of utilization. This requirement is not influenced by differences in absorption. Unfortunately, estimates of components of the factorial model are difficult to determine using conventional techniques. For example, the published figures for endogenous losses of copper in cattle vary by a factor of three (Smith et al. 1968, Suttle, 1974), and assessment of growth requirements of calves by a factor of six (Kirchgessner and Neesse, 1976).

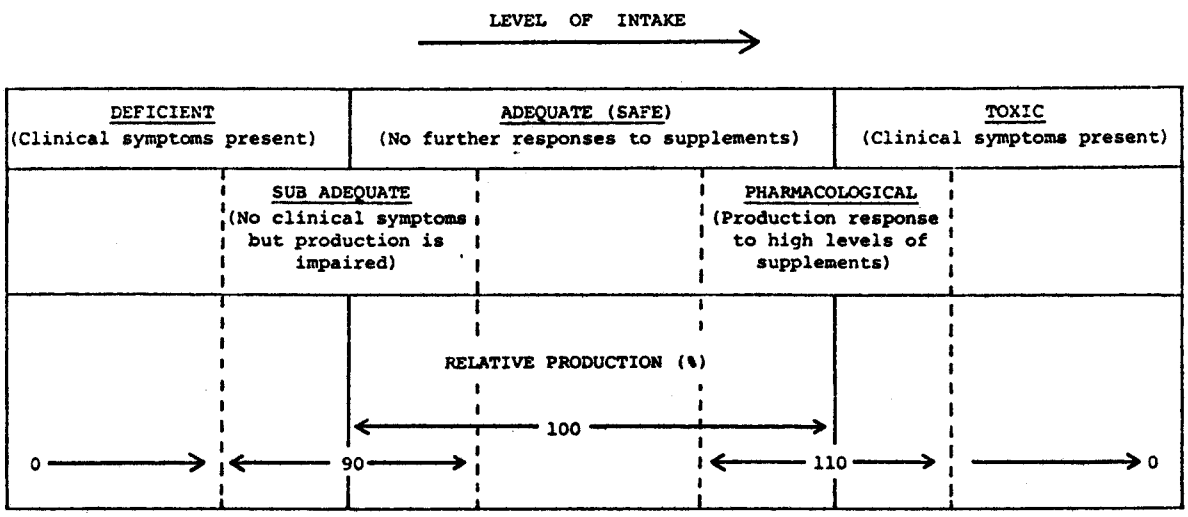
Suttle (in press) has attempted to overcome these problems by injecting deficient animals with a range of copper levels. He has estimated requirements for maintenance, growth and efficiency of

absorption of copper for growing calves. Hopefully, this technique will prove useful for other elements, but major technical problems remain to be overcome.

III. DETECTION OF DEFICIENCIES

Like requirements, deficiency can be defined in a number of ways. Referring to table 2, a severe deficiency is accompanied by pathological symptoms. Examples include white muscle disease in sheep and cattle in the case of selenium, enzootic marasmus for cobalt, and ataxia or swayback in lambs with copper deficiency. Blood and tissue levels of trace elements are generally reliable indicators of clinical deficiency states. However, unreliable analytical methods and the wide variability between animals makes these data of limited value in detecting subclinical deficiencies.

TABLE 2. Schematic respresentation of responses to various levels of trace element intakes in animals.



A subclinical deficiency is not characterized by any obvious pathological symptoms, and may show up as a 5 or 10 percent response in production to supplementation. Examples of this are becoming increasingly common. Spais and Papasteriadis, (1974) in Greece reported a 7% live-weight response to zinc supplements in cattle grazing pastures containing 20 to 30 mg/kg zinc. Egan (1972) in South Australia reported a 20% increase in lambing in response to zinc and manganese in ewes grazing pastures of 16-29 mg/kg zinc and 24 to 80 mg/kg manganese. Purser (personal communication) in Western Australia reported a 25% increase in liveweight gain in sheep in response to molybdenum supplements. The pasture contained levels of 0.1 mg/kg molybdenum in this experiment. In the Greek report, only 4% of cattle were showing clinical symptoms prior to supplementation. In the latter two cases, poor reproductive performance and ill thrift, respectively, were the only symptoms.

It is to be hoped that biochemical tests will be helpful in diagnosing subclinical deficiency states, but unfortunately they are currently only of limited usefulness. In many cases the relationship between dietary levels of the element and specific enzyme responses have not been established. For example, no metalloenzymes for tin, vanadium, arsenic or nickel have been reported in animals. In contrast, zinc is

known to be present in at least 59 pro - and eukaryote enzymes, and yet no zinc - containing enzyme has been shown singularly useful in diagnosing zinc deficiency in animals. Current evidence suggests the activity of thymidine kinase, a non zinc enzyme, most closely correlates with the growth depression associated with early stages of zinc deficiency (Prasad and Oberleas 1974), although the reason for this is not yet apparent. Henkin (1975) has suggested that the salivary protein, gustin, may be a useful indicator of zinc status in humans, but this has not been studied in animals.

The method for detecting cobalt deficiency in sheep may provide an insight into techniques which will prove useful for other elements. The method is based on knowledge of biochemical pathways requiring vitamin B_{12}, (cyanocobalamin), and consists of relating the level of urinary methyl malonic acid to the animal's B_{12} status (Gawthorne 1968). Similar techniques are routinely used for the detection of genetic diseases in humans, and may prove useful in screening for trace element deficiencies in livestock.

Selenium is also worthy of mention in this context, since recent reports show that pulmonary excretion of ethane and pentane is a sensitive indicator of *in vivo* peroxidation of ω6 and ω3 unsaturated fatty acids in rats, (Hafeman and Hoekstra 1977a,b). The usefulness of this technique for ruminants remains to be determined.

Returning to Table 2, a pharmacological response is indicated by the increase in production seen at relatively high levels of trace element intake. Examples include the 10% growth stimulation afforded by 250 mg/kg copper in the diet of pigs (Barber *et al.* 1955), and the 20% increase in the fibre diameter of wool of sheep receiving 50 or 100 mg of copper/day as an oral dose, compared with those receiving 5 mg/day (Palmer 1939). The highest dose level in the latter experiment was toxic (four out of six sheep died), but given the correct balance of copper and zinc, these sheep may have all survived.

The aetiology of these so called pharmacological responses is unknown. Kirchgessner *et al.* (1976a) may have provided a rational explanation of the effects observed in the pig, since they reported that high levels of copper and nickel ions stimulated pepsin activity *in vitro*.

IV. TRACE ELEMENTS AND GENETIC DISEASES

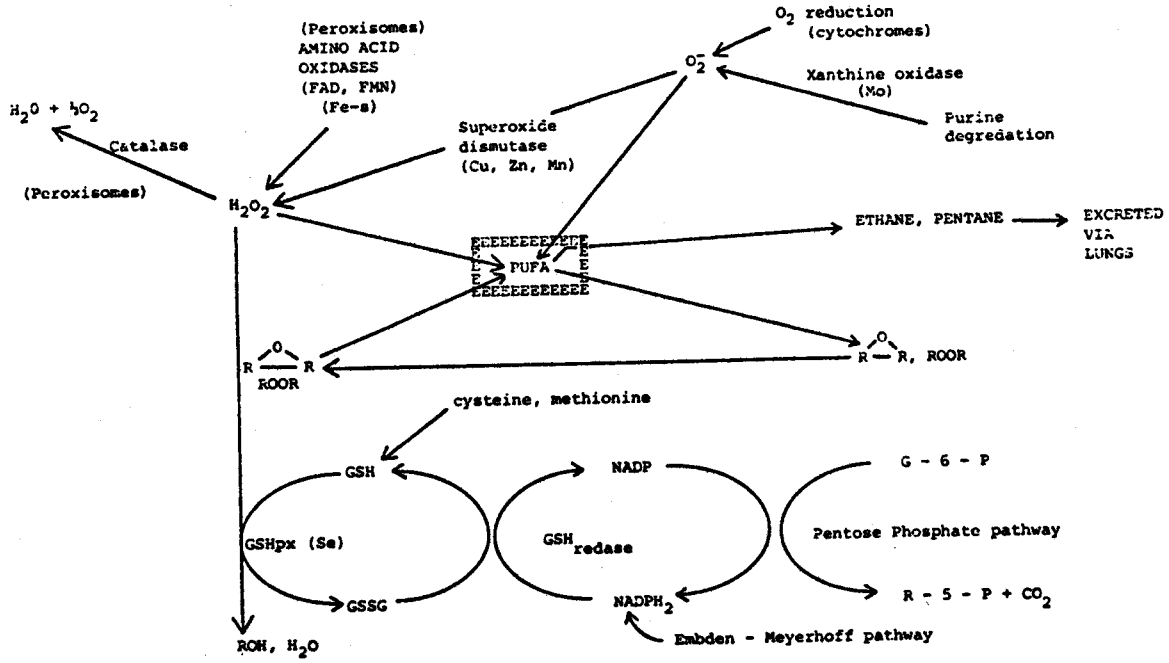
Discoveries of genetic diseases which are manifestations of poor absorption or utilization of trace elements have made scientists aware of the problems inherent in detecting deficiency states. This is proving to be especially important in cases where impairment *in vitro* or early post-partum may have irreversible effects on later production potential. For example, Piletz and Ganschow (1978) showed that female rats possessing the lethal milk gene (lmlm) possess 34% less zinc in their milk than normal rats. After 3 days on this milk, pups did not survive even if subsequently given zinc injections. However, zinc injections at birth, or transfer of pups to normal dams completely prevented the poor growth and mortality. This genetic defect was not characterized by a reduction in plasma zinc in dams or their pups, suggesting that the rate of transfer of available zinc from the intestine to the tissues was the critical factor in the deficiency syndrome.

Other genetic diseases shown to respond to high levels of zinc are acrodermatitis enteropathica in humans (Moynahan 1974, Nelder and Hambridge 1975), and adema disease in cattle (Flagstad 1976). The former disease was shown to be a defect in zinc absorption from synthetic or cows milk, whereas zinc in the mother's milk was shown to be readily absorbed. In adema disease of strain A46 friesians in Denmark, zinc absorption was impaired, and calves showed poor growth and impaired thymus development. These types of effects may explain some of the inconsistencies in the literature regarding field responses to zinc supplements. They also serve as examples of situations in which responses to trace elements are highly dependent on the timing and dose level of the supplement.

V. INTERACTIONS

An outstanding piece of scientific detective work in recent years was the discovery by Rotruck that GSHpx was a selenoenzyme (Rotruck et al. 1973). This discovery explained many of the observed interactions between poly-unsaturated fatty acids, vitamin E, sulphur amino acids and selenium (see figure 1). More recent work from the same laboratory firmly established the importance of vitamin E and selenium in protecting the cell against in vivo peroxidative damage (Hafeman et al. 1977a,b).

FIGURE 1. Schematic Diagram showing the biochemical interrelationships between selenium, vitamin E and sulphur amino acids.



These discoveries, together with that of McCord and Fridovich (1969) of the oxidant protective role of the copper-zinc-manganese enzyme(s), superoxide dismutase, has contributed much towards our understanding of the way in which mammalian cells regulate their metabolism in an oxygen environment, and the dependence of this regulation on a number of trace elements.

At the applied level, interactions between selenium, vitamin E and other elements may play a major part in the aetiology of white muscle disease and other selenium-responsive conditions. White and Somers (1977),

and Pope et al. (in press) have shown that increases in the dietary level of sulphur alters selenium metabolism and reduces selenium retention in sheep. The exact relationship between these effects and selenium requirements of grazing animals remains to be determined.

Copper has also been shown to interact with selenium in grazing (Hill et al. 1969, Thompson and Lawson 1970) and pen fed sheep (White et al. in press). In the latter experiment, the addition of only 10 mg/kg copper as copper sulphate to the diet of lambs resulted in a marked increase in blood and tissue GSHpx.

Other recent advances in our understanding of trace element interactions have been made by Dick et al. (1975) who demonstrated the existence of thiomolybdate anions in rumen fluid, and postulated their role as agents primarily responsible for the Cu-Mo-S interaction first observed by Dick in 1954. According to Mills et al. (in press) molybdenum-antagonized-copper-deficiency is strongly synergised by a variety of dietary sources of sulphur, and may be responsible for the development of copper deficiency in ruminants in many areas. Although the biochemical basis of the interaction is not completely resolved, it appears certain that thiomolybdate ions form a major part in the aetiology of Cu-Mo-S interactions.

Many other interactions have been reported which influence trace element availability in feedstuffs, especially those involving copper, iron and zinc, but these have been adequately covered elsewhere (Mills 1974; Waddell 1974 and Davies 1974, respectively).

VI. OUTLOOK

From reviewing this subject, it has become obvious how great the need is for more quantitative information about animal requirements and the biological availability of trace elements. I shall not attempt to place priorities on research, but the following areas require attention.

1. To refine techniques for the accurate and reproducible measurement of trace elements in feedstuffs and tissues. Much of the confusion surrounding requirements and diagnoses of deficiencies is due to the variability between laboratories in their ability to accurately analyse for trace elements.
2. To develop cheap and effective means of supplementing animals. This is especially important in Australia, where animal husbandry practices are not generally conducive to salt supplements.
3. To investigate trace element interactions and factors influencing the biological availability of trace elements in various feedstuffs.
4. To develop sensitive diagnostic procedures for detecting subclinical deficiency states.
5. To accurately estimate requirements of trace elements for maintenance, production and endogenous excretion in animals.
6. To elucidate biochemical functions of trace elements.
7. To study trace elements whose essential role is not established.

It is hoped that the reasons for these research needs have been adequately covered in the preceding discussion. Many of these areas are

not glamorous, and require repetitive experimentation with a variety of feeds and elements. However, the research must be done if we are ever to fully understand trace element nutrition and biochemistry in animals.

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