Predicting the risk of mineral deficiencies in grazing animals

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Problems relating to the anticipation and elimination of poor production due to mineral deficiencies in grazing livestock will be examined in this paper. Its concern is with subclinical problems rather than catastrophic clinical problems. The ways in which hypothetical models of animal requirements and real data on the responses of lambs to mineral supplements can be used to predict risks of deficiency will be demonstrated. In both cases mineral availability (the potential of the feed as a source of absorbable mineral) will be seen to play a key role.

In hierdie artikel word probleme in verband met die voorspelling en uitskakeling van swak veeproduksie as gevolg van minerale tekorte, ondersoek. Sorg word veral gegee aan subkliniese probleme voordat dit lei na kliniese katastrofes. Maniere waarop hipotetiese modelle van die behoeftes van diere asook werklike data oor die reaksies van diere op minerale byvoegings gebruik kan word om die risiko van tekorte te voorspel, word gedemonstreer. Minerale beskikbaarheid (die potensiaal van voedsel as 'n bron van absorbeerbare minerale) speel in albei gevalle 'n sleutelrol.

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Introduction

Problems relating to the anticipation and elimination of poor production owing to mineral deficiencies in grazing livestock are examined in this paper. Its concern is with subclinical problems rather than the catastrophic clinical problems which attended the establishment of pastoral systems in countries such as South Africa. The classical studies of Sir Arnold Theiler and his associates on aphosphorosis on veld pastures demonstrated just how much can be achieved by the careful study of clinical responses to supplementation: their work still provides the clearest demonstration of the low availability of P in rock phosphate compared with other sources of the mineral (Theiler, du Toit & Malan, 1938). I will demonstrate the ways in which hypothetical models of animal requirements and real data on the responses of lambs to mineral supplements can also be used to predict risks of deficiency: in both cases mineral availability (the potential of the feed as a source of absorbable mineral: Suttle, 1986a) will be seen to play a key role.

Alternative strategies

The three conventional approaches to the anticipation of mineral deficiencies are illustrated in Figure 1. They involve analysis of the mineral concentrations in soils, pastures or animals. In each case there have been movements from the simple measurement of total mineral concentrations to the assessment of that fraction which is available for use. One might expect that the closer one moved to the functional sites of the minerals in animals the better would the predictive value of the biochemical index be. The reality is that risk of lost performance cannot be accurately predicted by any of the conventional strategies. Each strategy relies heavily on 'accumulated wisdom' from 'the field' in the form of loose associations between mineral status and animal



Figure 1 An illustration of the conventional approaches to the prediction of risks of mineral deficiencies in grazing livestock (1, 2, 3), their reliance on accumulated wisdom and theoretical calculations of animal needs (4) and their need for refinement through observed relationships with real responses to specific supplements (5)

health. The first and second approaches usually involve a fourth stage in which observed or predicted trace element concentrations in feeds are compared with estimates of animal needs often presented in the form of convenient tables of requirements. Large land masses have been characterized as multiply deficient in minerals by reference to critical threshold concentrations for minerals in the blood or tissues or to tables of dietary requirements (e.g. McDowell, Conrad & Ellis, 1984). How reliable are these approaches? Table 1 gives normal limits for plasma P and estimates of P requirements in growing lambs from different authorities. The differences between them are marked and would be there whatever mineral was cited. The differences reflect either differences in interpretation of the same experimental data or differences in the margin of safety

Table1Differencesbetweensourcesinrecommendations on the P requirement of growinglambs and the serum P concentrationthat wouldindicate deficiency in the animal

P requireme 40\kg lamb 0,2 k	ent (g/d) of growing at g/d	Threshold for hypoj in shee (Serum Р п	Threshold for hypophosphataemia in sheep (Serum P mmol/l)		
Source	Standard	Source	Standard		
INRA (1978)	3,5	Doxey (1977) (Scotland)	0,90		
NRC (1985)	3,1	MAAF (1978) (England & Wales)	1,13		
ARC (1980)	2,3	Kaneko (1980)	1,56		

INRA — Institute National de la Recherche Agronomique (France) NRC — National Research Council (USA)

ARC — Agricultural Research Council (UK)

MAAF — Ministry of Agriculture, Fisheries and Food

which the authority saw fit to include. Clearly the assessment of risk of mineral deficiency will depend upon whose standard forms the basis for comparison.

New factorial models for Ca and P requirements

A recent reassessment of Ca and P requirements for ruminants (TCORN, 1988) has shown just how fragile the basis of many factorial estimates were. The basic model remains the same involving the summation of maintenance (M; equivalent to endogenous loss) and production (P) demands and their division by an absorption coefficient (A) i.e. dietary requirement = (M + P)/A. The model has had to become flexible to allow for fluctuations within and correlations between its components.



Figure 2 Relationships between dry matter intake (DMI) and the faecal endogenous loss of (Ca E_{Ca} (g/d))as observed in lambs or derived from published data on cattle. The cattle data of Visek, Monroe, Swanson & Comar (1953) gave a relationship before scaling for live mass in which $E_{Ca} = 1,068$ DMI - 0,92 (r = 0,78)

Dry matter intake, diet type and the maintenance requirement

The maintenance requirement for a mineral is very important under pastoral conditions such as those of the South African veld where for a whole season it may constitute the sole requirement. The need for maintenance had always been regarded as a constant fraction of body mass, reflecting for the most part the minerals which pass through and from the mucosa of the alimentary tract and escape reabsorption. The constancy of the maintenance requirement was first challenged by Braithwaite (1982) who showed that the faecal endogenous loss of Ca in sheep depended upon how much food (given as a mixture of roughage and concentrates) an animal of a certain body mass consumed (Figure 2). While there is a rough relationship between body size and appetite, the onset of lactation or the feeding of highly digestible feeds will change the relationship. Retrospective examination of data for cattle on a mixed diet suggests that their maintenance needs will also be affected by dry matter intake (DMI; Figure 2). Instead of a constant there should be scope for a three-fold variation in the maintenance requirement for Ca.

The faecal endogenous loss of P is also believed to increase by similar proportions with changes in DMI (TCORN, 1988). One implication of this is that the more of a P-deficient diet an animal consumes, the more severe will the consequences of that deficiency be. Indeed the anorexia of P deficiency may be part of an adaptive response which minimizes the severity of deficiency on such diets rather than a simple consequence of deficiency.

Previously, the maintenance requirement for minerals was taken to be quite independent of the type of diet being fed. However, the maintenance requirement for P is probably higher on loose roughage than on pelleted diets. The point is not easily demonstrated because the principal source of obligatory loss, salivary P secretion, is also a major route for excreting surplus P until urinary P excretion takes over at very high P inputs. By selecting from the findings of Field, Woolliams & Dingwall (1984) the data for three sets of identical lambs which excreted P in their urine, the increased loss of absorbed P via the faeces on loose roughage compared with pelleted diets is demonstrated by a substantially higher threshold for urinary P excretion (Figure 3). Increased salivary flow of loose roughage diets provides an obvious explanation for the roughage effect but the extent to which it is influenced by DMI and P supply has yet to be clarified.

The maintenance requirement for other minerals may similarly be related to DMI and it is noteworthy that experimental studies in this field have generally used low intakes of highly digestible diets, sometimes in pelleted form: these give minimal stimulation to salivary flow and may therefore give minimal estimates of the mineral needs of grazing animals for maintenance.



Figure 3 Urinary excretion of absorbed phosphorus (P) was consistently lower on loose roughage than on pelleted diets in three sets of chimaera-derived lambs (excluding Set 2 from Field, *et al.* 1984) providing indirect evidence of greater partition to faecal endogenous excretion on the loose roughages

Requirements of Ca and P for growth

The requirements of minerals for growth, like those for maintenance, have commonly been regarded as constants per unit body mass. Langlands & Sutherland (1968) were the first to question this, but their data showing decreases in Ca and P/kg body mass with age did not change the modelling approach, despite clear support in the literature (Kellaway, 1973; Grace, 1983). The decline in requirement with the approach to maturity is readily explained by the decreasing contribution of bone to mass gain in the fattening animal.

What happens at the other extreme in the suckling animal? It is hard to dispel thoughts of milk as the ideal source of minerals, particularly for bone growth, but the reality is that it cannot give rise to a fully mineralized skeleton. Ewe's milk for example is converted to body mass at 5kg/kg (fresh basis): because it contains only 1,6 g Ca/kg it cannot supply the growth requirement of 12 g Ca/kg and there thus is a 33% shortfall.

Requirements of Ca and P for milk production

Another popular concept which must be revised is that the skeleton is a perfect reserve of Ca and P in times of dietary shortfall, especially with the onset of lactation. Milk contains Ca: P in a ratio of 1,3:1,0, while the skeleton holds Ca:P in a ratio of 2,1:1,0 and cannot release one without the other in stoichiometric proportions. Bone mineral does not constitute a balanced supply of Ca and P for milk production and the possible importance of this in the aetiology of milk fever should become apparent in the discussion of the control of Ca absorption.

Availability of calcium

The absorption coefficient for a mineral used in determining dietary requirement is of crucial importance. The decision by ARC (1980) to adopt a maximum attainable value of 0,68 (i.e. the potential availability of Ca) reduced the dietary requirement for Ca by 50% from that of a previous working party (ARC, 1965) who used a value of 0,40. The change caused consternation in the UK feed industry and was rejected in their interests (Todd, 1983). The ARC's (1980) decision arose from a new concept that Ca was absorbed according to need and that the low average value for 'availability' in the literature merely reflected the fact that diets containing roughage would oversupply all but the most demanding animals with Ca: such animals (fast growing or high milk-yielding) had rarely been used as the experimental subjects when Ca absorption was being measured.

The ability of the lactating ewe to attain such high levels of Ca absorption has been questioned by Braithwaite (1983) who found absorptive efficiencies as low as 35% in ewes on low Ca intakes. He suggested that the adaptive mechanisms may be slow to take effect in early lactation and that the ARC (1980) had underestimated Ca requirements. However, another explanation involving the unbalanced nature of the skeletal reserve can be offered for his unexpected results. His ewes were fed diets low in P and they became hyphosphataemic, despite resorbing much P from bone. They remained normocalcaemic, however, presumably because the resorbed bone, with Ca:P at 2,1:1, provided more than enough Ca to meet the dietary deficit. Only a drop in plasma Ca would trigger the parathyroid hormone-mediated, adaptive increase in Ca absorption. Put another way, the ability of the animal to absorb Ca according to need is contingent upon an adequate supply of dietary P and P supply will influence the hormonal adaptation to onset of lactation.

There may even be technical and physiological reasons why conventional balance methods will never give results for Ca availability which are appropriate to grazing animals. It is an accepted feature of human mineral metabolism that confinement induces demineralization of the skeleton. If the same response occurs when animals are confined in metabolism crates, values obtained from them are likely to underestimate those which would be found in free-ranging animals.

We are left knowing virtually nothing about the availability of Ca in natural foods because experimental conditions have rarely tested the potential of feeds to the full (TCORN, 1988).

Availability of P

The absorption of P is not influenced by the animal to anything like the extent that obtains for Ca. This has one advantage in that the potential of feeds as sources of absorbable P can be measured over a wide range of P intakes. There remains a need to use radioisotopes to distinguish unabsorbed dietary P from unabsorbed endogenous (largely salivary) P, and this has restricted the acquisition of data. There is little reliable information in the literature from which to rank feeds other than that provided by Field, et al. (1984) (Table 2). In showing that genetic influences on P absorption (i.e. differences between chimaera) were as great as differences between feeds, they showed that isolated studies involving small samples of an animal population are likely to yield biased estimates of P availability. Within-animal comparisons are needed to give unbiased

Table 2 Differences between sets of identical, chimaera-derived sheep in the mean efficiency with which they absorb phosphorus from foodstuffs can be as large as the variation between foodstuffs (from Field, *et al.*, 1984)

Chimaera set	Mean	Mean absorption coefficient for dietary P			
	Rice bran	Grass hay	Soya bean	Fish meal	
1	0,63	0,75	0,69	0,84	
2	0,54	0,67	0,55	0,61	
3	0,68	0,78	0,75	0,90	
4	0,70	0,80	0,77	0,89	

estimates, and none has been made. Field, *et al.* (1984) gave the following availabilities for single samples of concentrate feeds: white fish meal, 0,80; soya bean meal, 0,72; maize gluten, 0,71; barley, 0,78; wheat, 0,78; rapeseed meal, 0,70 with an overall mean (*sd*) of 0,79 (0,04). The mean (*sd*) availability for six dry roughages, 0,68 (0,07), was lower and there was wider variation suggesting a need for further study. Although values higher than 0,9 were recorded for P availability in lucerne in the early days of isotope dilution techniques (Lofgreen & Kleiber, 1953), they may be biased.

Animal variation in P absorption

Genetic variation in P absorption is probably manifested in cattle as well as sheep (Field & Suttle, 1979) and could be exploited as an alternative to P supplementation, which merely benefits and preserves the least efficient users of dietary P in the population. Given the endemic nature of P deficiency on veld pasture and the diversity of bovine genotypes in South Africa there would appear to be ample scope for controlling P deficiency by selection.

Genetic variation creates problems in calculating and using P requirements because the average requirement, calculated with an average P availability, will be too little for one half and too much for the other half of the population. Given the immaturity of the knowledge which underlies factorially derived requirements, there are obvious limits to which their predictions can be relied upon to assess risks of deficiencies for P, Ca and other minerals in the field: the variation between standards (Table 1) is a useful reminder of their vicissitudes.

Reliance on observed mineral concentrations in soils, plants or animals and incidence of deficiency offers no immediate advantage over tables of requirement in the accuracy of predicting risk of mineral deficiency. The literature is full of examples of poor correlations between biochemical indices and animal performance and interpretations vary (c.f. Table 1).

Response to supplementation

An alternative strategy (Figure 1(5)) is to accumulate comprehensive data on the natural mineral environment together with production responses when mineral supplements are fed to some groups but not others. Such information provides an objective basis on which to assess the criteria of adequacy in soil, plant and animal that are haphazardly employed at present. If, in addition, approximate estimates of food intake and mineral availability could be made, such field studies would provide more reliable estimates of mineral requirement than anything obtained from unnatural experimental conditions and mathematical models based on their results.

An example of this approach is afforded by some coordinated dose-response trials in lambs on improved pastures in Scotland which are assessing the importance of, and means for, predicting the growth-retarding effects of Cu, Co and Se deficiencies (Suttle, Wright, MacPherson, Harkess, Halliday, Millar, Phillips & Evans, 1986). Two new biochemical criteria of deficiency in animals, erythrocyte superoxide dismutase (ESOD) for Cu (Suttle & McMurray, 1983) and plasma methylmalonic acid (MMA) for Co (McMurray, Rice, McLoughlin & Blanchflower, 1985) are being evaluated. EDTA-extractable soil Mo is also being assessed as a predictor of antagonism towards Cu. One statistically significant response to supplementation may be sufficient to generate an estimate of the mineral requirement for normal growth with respect to the best criterion, measured in soil, pasture or grazing animal, which is closer to the truth than a hypothetical factorially derived requirement.

A successful application of the dose-response approach, albeit within the context of a single farm, is being tried using a unique set of data showing genotype \times environment interactions affecting susceptibility to Cu deficiency. The example also illustrates the importance of being conscious of the possibility of hitherto unknown consequences of mineral deficiency and the scope for genetic selection in controlling them.

The three-year study is described in detail elsewhere (Woolliams, Suttle, Woolliams, Jones & Wiener, 1986a; Woolliams, Woolliams, Suttle, Jones & Wiener, 1986b). Briefly, hypocuprosis was induced by introducing ewes of a vulnerable breed, Scottish Blackface, to improved hill pastures. Slightly increased herbage Мо concentrations in the presence of high herbage S were probably responsible for lowering Cu availability (Table 3, after Suttle, 1986b). Hypocuprosis was manifested in the conventional ways (swayback, anaemia, diarrhoea and growth retardation) but also in a new form which exceeded the others in economic importance, increased susceptibility to fatal infections. All the signs were greatly reduced by Cu treatment. Genetic involvement was proven by selecting for high (H) or low (L) plasma Cu within an inbred cross between the Scottish Blackface and a breed resistant to Cu deficiency, the Welsh Mountain. After four generations, the parental, Cu-sensitive traits were apparent with the line, like Blackface lambs, having poor survival and growth unless supplemented (Table 4).

The study involved large numbers of lambs (470/year) and a wide spread of Cu status against which to seek responses to Cu supplementation. The L and H lines in

Table 3 Effects of small changes in the molybdenum and sulphur concentrations in herbage on the availability of its copper to grazing sheep (from Suttle, 1986b)

Cu availability (i.e. true absorption coefficient)			
Mo (mg/kg DM)	1	3	
S(g/kg DM)		· · · · · · · · · · · · · · · · · · ·	
1	0,045	0,020	
2	0,036	0,015	
4	0,015	0,003	

Table 4 Survival to 4 weeks and growth to 6 weeks of age in lambs from lines selected for low (L) or high (H) plasma copper for five generations (from Suttle, Jones, Woolliams, & Woolliams, 1987): half of their dams were given a Cu supplement (+ Cu) in mid-pregnancy and they were separated into early and late lambing sets for rotational grazing (sets 1 and 2 respectively)

	Set		L	Line			
]	L		н		
		0	+Cu	0	+Cu		
Survival to 4 weeks (as							
proportion of lambs born	1	0,747	0,961	0,880	0,885		
	2	0,656	0,941	0,826	0,833		
Live mass ^a at 6 weeks (kg)	1	12,3	12,4	12,4	13,2		
	2	14,1	15,0	15,2	16,8		

^a Approximate se 0,47



Figure 4 Sensitivity of resistance to infection and growth loss from not receiving a Cu supplement in lambs over 6-24 weeks as measured by plasma Cu concentrations and erythrocyte superoxide dismutase activity (SOD) at the beginning of the risk period (from Suttle, *et al.*, 1987)

particular provided an ideal opportunity to predict Cu responsiveness in each affected function (disease resistance, growth, haemoglobin synthesis) without the complication of breed differences unrelated to Cu status. The outcome is illustrated in Figure 4. Some prediction equations are given in Table 5. The exercise shows that average plasma Cu concentrations must fall well below the currently accepted norm (9,0 μ mol/l) before growth is compromised and also that disease resistance is an equally sensitive function of Cu status. The new index of Cu status, ESOD, clearly has predictive merit for growth retardation (Figure 4).

Individual variation in mineral responsiveness is particularly important when responsiveness is a matter **Table 5** Prediction equations relating probability of death and response in live mass or haemoglobin to Cu supplementation between 6 and 24 weeks to plasma Cu concentration (P μ mol/l) or erythrocyte superoxide dismutase (ESOD) (U/ml blood) at 6 weeks of age in lambs on improved hill pasture (from Suttle, *et al.*, 1987)

	Regression coefficient	Intercept	r
Probability of			
death (logit value)	-0,48P	-0,70	$(P < 0.05)^{a}$
	-0,00815 _{ESOD}	-0,438	$(P < 0.05)^{a}$
Growth response (kg)	$-1,506P_{log}$ 10	2,19	0,62
	-1,58ESOD _{log 10}	9,89	0,89
Haemoglobin	Ū.		
response (g/dl)	$-3,377P_{log}$ 10	2,31	0,96
	-3,863ESOD _{log 10}	13,02	0,67

^a Correlation coefficient statistically inappropriate

of life or death. Suttle (1986c) has illustrated the changing spectrum of responsiveness which is likely to occur when animals pass from a stage of depletion (loss of reserves), to deficiency (decreases in concentrations in transport pools) and dysfunction (decrease in mineral-dependent enzyme activity). The predictive ideal must be to anticipate the likelihood of any individual showing 'immune dysfunction' and in these circumstances the number sampled must be large ($\ll 10$): indeed, variation about the mean becomes more significant than the mean itself. Ideally no individual must be left at risk of dying from infections preventable by Cu supplementation. Given the error in the prediction equations, the 97,5% confidence limit (2 sd 's below mean) is probably as far as one should go in predicting individual risk.

The approach to predicting other mineral risks will depend on the importance of disease susceptibility as an early consequence of deficiency. There is evidence that Co deficiency and Se deficiency impair the responses of phagocytes and lymphocytes in vitro (Paterson & MacPherson, 1987; Turner, Wheatley & Beck, 1985) but as yet no evidence that they increase the incidence of chronic or fatal infections in vivo. If growth retardation is the only consequence of economic importance, then the small size of the potential benefit must be taken into account. Fraser (1982) reports that growth responses to Co supplementation occur in New Zealand when the mean vitamin B₁₂ concentration in lamb plasma falls below 250 pg/ml. Paynter, Anderson & McDonald (1979) found that growth retardation occurred when mean activity of the seleno-enzyme, glutathione peroxidase (GSHPx), fell below 30 U/g Hb (measured at 37°C). The maximal growth reduction was only 10% however. In the 'three year' study, a Se deficiency was also detected: a mean GSHPx activity of only 16 U/gHb was associated with a growth reduction of 17% (Suttle, Jones, Woolliams, Woolliams & Weiner, 1984). In each **Table 6** Tendency for growth responses to copper and selenium to be more than additive when given together to Scottish Blackface (B) or Welsh Mountain (W) lambs on pastures deficient in both elements (Suttle, Jones, Woolliams & Woolliams, 1984)

Live mass gain advantage between 12–24 weeks (kg)						
Cu response			Se response			
Se treatment	В	w	Cu treatment	В	W	
0	2,9	0,3	0	0,5	1,2	
+	3,6	1,0	+	1,2	1,9	

case the growth response is probably attributable to a minority in each group with the lowest trace element status at important functional sites such as the liver.

The three year study also illustrates the importance of allowing for the possible influence of multiple deficiencies. The Se treatment was part of a 2 \times 2 experiment which also involved a Cu treatment. The table of responses (Table 6) shows that although the Cu \times Se interaction was not statistically significant, for both breeds the response to each element was greater when the other was also given. Such factorial designs are probably superfluous under most field conditions because significant interactions with only one *df* are probably unattainable. The procedure should therefore be to omit only one element at a time in looking for subclinical responses (e.g. Suttle, *et al.* 1986).

With small potential growth responses, it would be unwise to place undue emphasis on the attainment of statistically significant responses on individual farms because economically worthwhile responses to cheap forms of supplementation may be reached before they become statistically significant. The size of the response, whether significant or not, should correlate with trace element status on a between-farm basis and form a basis for predicting the possibility of lost production.

Conclusions

There is still a long way to go when it comes to controlling mineral deficiencies. Although methods of supplementation are rapidly improving, the major constraint is knowing when supplements should be applied. That decision must be governed partly by the frequency and costliness of the impaired function and it is important to establish whether mineral deficiencies other than Cu deficiency directly affect survival in grazing animals. Improvements in the biochemical diagnosis and anticipation of deficiencies are more likely to come from comprehensive field studies rather than narrow laboratory studies. With its wealth of naturally occurring deficiencies, South Africa provides an excellent opportunity for major advances in this important area of nutritional investigation.

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