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Recent developments in the detection and treatment of vitamin B_{12} deficiency in sheep and cattle at pasture

G.J. JUDSON, P.J. BABIDGE, Z.H. CHEN[1] AND L.N. SANSOM[1]

Livestock Systems, South Australian Research & Development Institute, 33 Flemington Street, Glenside South Australia, 5065.

ABSTRACT

Marginal Vitamin B_{12} deficiency is economically more important than a severe deficiency of the vitamin since the former can go unrecognised by the stockowner and hence uncorrected. This paper discusses recent work in this area by the authors, and reviews diagnostic procedures for the detection of Vitamin B_{12} deficiency in ruminants and developments in the treatment of this deficiency.

Keywords: cobalt; vitamin B_{12}; ruminants; polymer-based animal remedies.

INTRODUCTION

It has been stated that a pasture cobalt (Co) level of 0.11 mg/kg DM is required to maintain optimum Vitamin B_{12} status of livestock (Underwood and Suttle, 1999). Optimum status is desirable not only to meet the requirements for growth and reproduction but also to ensure immune competency and provide adequate reserves to cover periods of transient deficiency. Recent evidence with cattle fed semi-synthetic diets suggests that the Co requirement for maximum growth of young bulls was 0.12 mg/kg Co and that an adequate level based on biochemical indicators of Vitamin B_{12} status was 0.20 mg/kg (Stangl *et al*., 2000). The higher requirement may in part be due to the feeding of high-energy diets reported to depress Vitamin B_{12} production in the rumen and favour the synthesis of analogues (SCA, 1991).

DETECTION OF DEFICIENCY

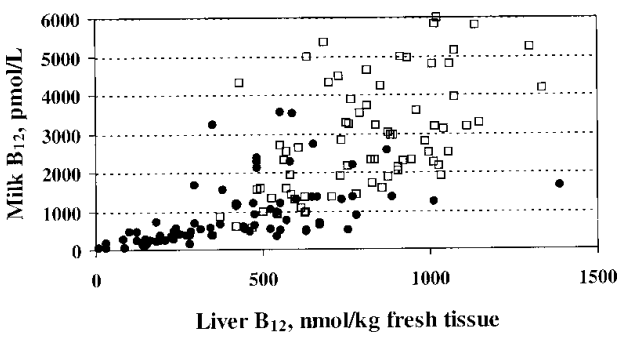
Tissue vitamin B_{12} concentrations

In sheep plasma Vitamin B_{12} levels are responsive to dietary intake of Co, while liver levels indicate Vitamin B_{12} reserves and respond relatively slowly to changes in Co intake (Sutherland, 1980). In New Zealand the suggested thresholds for Vitamin B_{12} adequacy are 500 pmol/L plasma and 375 nmol/kg fresh liver in sheep (Clark *et al*., 1989) and 220 nmol/kg fresh liver in cattle (Gruner, 1999). No value was given for cattle plasma because of the difficulty of assay. These values are similar to those suggested for use in Australia apart from the liver Vitamin B_{12} value for sheep, which is similar to that for cattle (SCA, 1990).

Technical problems have been encountered in the assay of Vitamin B_{12} in cattle plasma using radioisotope assay procedures. The accuracy of the assay depends on the separation of the cobalamin from transcobalamins and on the type of binding protein chosen. It was found that some procedures did not release all the Vitamin B_{12} from serum binders or denature all binders in the serum. Our technique, using a commercially available kit, eliminates residual binding but plasma values in cattle at risk from Co deficiency are low (< 50 pmol/L) and non-responsive to changes in Co intake (Babidge, 1992; Judson *et al*., 1997b). It has been suggested that some procedures designed to free Vitamin B_{12} from transcobalamins in cattle plasma may also denature Vitamin B_{12} to analogues (Price *et al*., 1993). Recent studies in Germany indicate that plasma Vitamin B_{12} values associated with Co deficiency in cattle, as indicated by depressed growth rates and metabolic dysfunction, varied from about 100 to 150 pmol/L (Stangl *et al*., 2000). The radioisotope assay technique used was a commercially available kit. Evaluation of this kit showed that the conditions employed only partially removed Vitamin B_{12} from transcobalamins resulting in higher apparent Vitamin B_{12} concentrations (Babidge, unpublished findings).

Milk may prove an alternative to plasma as a sample for the assessment of the Vitamin B_{12} status of lactating cows. Milk Vitamin B_{12} values below 300 pmol/L were associated with inadequate liver Vitamin B_{12} reserves (<200 nmol/kg fresh weight) in beef cows at pasture (Judson *et al*., 1997a). Preliminary studies with dairy cows at different stages of lactation have confirmed that adequate liver Vitamin B_{12} reserves were associated with milk Vitamin B_{12} values above 300 pmol/L (Figure 1).

FIGURE 1. Relationship between liver Vitamin B_{12} and skim milk Vitamin B_{12} concentrations in beef (●) and dairy (❑) cows. The relationship is described by: $y = 206.5\ e^{0.003x}$ ($R^2 = 0.66$).



Metabolic indicators

Markers of metabolic disturbance offer promise of detecting early stages of the deficiency that are missed by measures such as productivity responses to supplementation. The vitamin is involved in energy metabolism, being a cofactor of methymalonyl CoA

[1] School of Pharmacy and Medical Sciences, University of South Australia, North Terrace, Adelaide, South Australia, 5000.

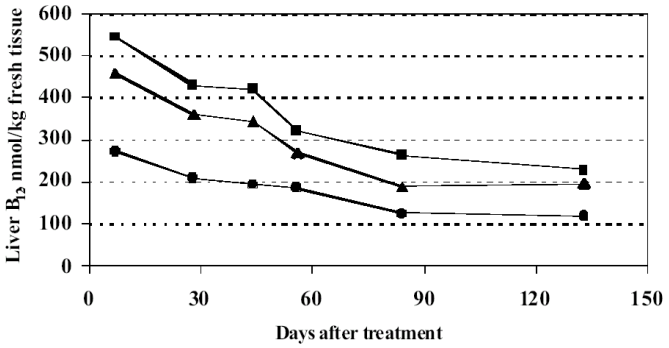
mutase which catalyses the conversion of methylmalonic acid (MMA) to succinate: MMA arises largely from ruminal propionate, a major gluconeogenetic precursor in ruminants. It is also a cofactor of methionine synthase, donating methyl groups for one-carbon metabolism during the synthesis of methionine from homocysteine.

The accumulation of MMA in plasma precedes clinical signs of Co deficiency in sheep (O'Harte *et al*., 1989; Rice *et al*., 1989) and cattle (Paterson & MacPherson, 1990) and is of value in detecting marginal Vitamin B_{12} deficiency in livestock with a functioning rumen. However, a reference range for plasma MMA needs to be established based on growth responses to Vitamin B_{12} supplementation. Dysfunction in methylation due to Co deficiency has been found in sheep to raise the concentration of homocysteine in plasma, formiminoglutamatic acid in urine and sulphur-adenosyl methionine in liver (Underwood & Suttle, 1999) and in cattle, to raise plasma homocysteine concentrations and lower liver folate concentrations (Stangl *et al*., 2000).

Failure in propionate metabolism provides an earlier warning of Co deficiency than a failure in one carbon metabolism as indicated in sheep based on plasma MMA levels and plasma urocanate, a precursor of formiminoglutamate (Price, 1991) or plasma homocysteine levels (Vellema *et al*., 1999). Plasma α-leucine levels also decline in early stages of Vitamin B_{12} deficiency in sheep and cattle (Babidge, 1993). Leucine 2,3-aminomutase, which catalyses the synthesis of α-leucine from β-leucine, is probably Vitamin B_{12} dependent and has been found in sheep liver (Poston, 1976).

Exposure to nitrous oxide (N_2O) is known to induce changes similar to vitamin B_{12} deficiency in various species. The major effect of N_2O exposure in sheep and cattle was a rapid inactivation of methionine synthetase activity in the liver, with raised homocysteine levels in plasma, and urinary excretion of formiminoglutamatic acid (Babidge, 1993). The other B_{12} dependant enzyme, methylmalonyl CoA mutase was not affected, with plasma MMA levels remaining low. Plasma α-leucine decreased only in sheep. This may be due to species differences in the relative flux in formation of α-leucine via the α- or β keto pathways. It appears that N_2O exposure could be useful for studying the effects of B_{12} deficiency, particularly on the methionine synthetase pathway.

FIGURE 2. Response in liver B_{12} concentrations in untreated sheep (●) and in sheep given 2 mg (▲) or 4 mg (■) of Vitamin B_{12} (80% hydroxocobalamin, 20% cyanocobalamin). Covariate - adjusted mean values are for 6 sheep. The least significant difference for all comparisons (P=0.05) was 83.7 nmol/kg fresh tissue.



TREATMENT AND PREVENTION OF DEFICIENCY

In Australia commonly accepted strategies of prevention are Vitamin B_{12} injections to the young and Co pellet therapy in weaned animals for long term protection. In Co deficient areas, the young animal is particularly susceptible to Vitamin B_{12} deficiency, being at risk from about one month of age (Judson *et al*., 1981; Grace, 1999a). Intraruminal Co pellets with grub screws, to reduce salt deposition on the pellet, are not recommended in New Zealand (Grace, 1994) because of risk of regurgitation of the pellet and of potential damage to processing equipment from metal objects retained in the offal. The poor retention of pellets has not been our experience in Australia with Merinos or British based breeds of sheep.

Vitamin B_{12} injections

A major limitation with the aqueous Vitamin B_{12} product, usually hydroxocobalamin or a mix of hydroxocobalamin (80%) and cyanocobalamin (20%) is the short duration of effect. When given to young sheep a 1 or 2 mg dose provides about 1-3 months protection (Judson, 1996; Grace *et al*., 1998) although in calves a single injection of 2 mg Vitamin B_{12} prevented reduced growth rates for up to 5 months (Judson *et al*., 1981). The vitamin is relatively non-toxic but we have found with sheep that larger doses are unlikely to increase the period of protection against Co deficiency (Figure 2).

The short duration of effect of the Vitamin B_{12} is due to rapid mobilisation from the injection site and rapid renal clearance. Young sheep given either 2 mg hydroxocobalamin or 2 mg cyanocobalamin excreted about 45% and 70% of the respective doses within 6 hours of treatment (Judson, 1996). The hydroxocobalamin, which was less readily excreted, was more effective in raising liver Vitamin B_{12} reserves than the cyanocobalamin.

A depot Vitamin B_{12} injection was developed for use in humans. It is a cyanocobalamin-tannin complex suspended in a sesame oil containing aluminium - monostearate gel. Studies with this product found that it was no more effective than the aqueous preparation of Vitamin B_{12} in raising the Vitamin B_{12} status of lambs at pasture (Judson *et al*., 1988) or penned sheep fed a cobalt deficient diet (Judson, 1996).

Attempts to develop a depot Vitamin B_{12} that was slowly mobilised from the injection site resulted in the synthesis of five monoesters and one diester of Vitamin B_{12} (Chen, 1999). These esters had slow *in vitro* hydrolysis rates and were designed to slowly release active Vitamin B_{12}. The esters contained side chains of varying carbon chain length. Evaluation of different doses of the esters in various carriers in sheep showed that the release rates *in vivo* were either too rapid or too slow to provide adequate Vitamin B_{12} over a 6 to 8 month period (Chen, 1999) but did exhibit a longer release profile as a function of hydrophobicity.

Lactide-glycolide copolymers, which are biodegradable, have been developed for use in controlled release formulations (Lewis, 1990). The polymer can be modified to encapsulate Vitamin B_{12} (Grace & Lewis, 1999; Chen, 1999). Studies in New Zealand with microspheres of the Vitamin B_{12}-polymer suspended in peanut oil have shown that single doses of 3 to 6 mg Vitamin B_{12} to lambs, and 12 to 24 mg Vitamin B_{12} to calves, were effective in raising Vitamin B_{12} status of lambs for up to 8 months, and of calves at least 3-4 months (Grace, 1999b; Grace & Sinclair, 1999; Grace & West, 2000a,b). Our studies with an aqueous and pellet forms of the Vitamin B_{12} microencapsulated with lactide/glycolide copolymer confirmed the long efficacy in sheep when using single doses of 2-3 mg Vitamin B_{12} (Chen, 1999; Judson *et al.*, 2000). Unfortunately, the Vitamin B_{12}-polymer complex has a limited shelf life in aqueous carriers because hydrolysis releases the soluble form of the vitamin. Using a non-aqueous carrier or forming it into a pellet can extend the shelf life.

Intraruminal boluses

Intraruminal boluses commercially available in Australia for prevention of Co deficiency include the high density Co pellet for sheep and cattle and a large multi-trace nutrient bolus for cattle. The decrease in the cobaltic oxide content of Co pellets from 60% to 30% has resulted in a revaluation of these for livestock (Judson *et al*., 1995; 1997b). These studies showed that liver Vitamin B_{12} reserves were raised for 1 to 3 years in sheep given one 10 g pellet and for about 1 year in cattle given one 30 g pellet and 2 years in cattle given two 30 g pellets. The longevity of the pellet appears to be affected not only by the Co content but by differences in the physical characteristics of the Co oxide or other constituents of the pellet (McFarlane *et al*., 1990). Adequate release of Co in the rumen is important not only for the synthesis of Vitamin B_{12} but in many areas of southern Australia, for the prevention of phalaris staggers.

In large areas of Australia livestock are at risk from both Co and selenium (Se) deficiency. Preliminary studies have been undertaken to investigate the efficacy of an intraruminal 30 g pellet containing about 30% cobaltic oxide and 10% elemental Se as a means of preventing Vitamin B_{12} and Se inadequacy in heifers at pasture (Judson and McFarlane unpublished findings). It was found that the combined pellet raised liver concentrations of Vitamin B_{12} for at least 280 days and Se for at least 91 days but these increases were not as marked as the increases obtained with the conventional treatment of one 30 g pellet containing 30% by weight cobaltic oxide and two 30 g pellets containing 10% by weight elemental Se (Table 1).

TABLE 1. Response in covariate-adjusted mean concentrations of Vitamin B_{12} and selenium in liver of heifers given different oral supplements. All heifers received one 30 g grub screw. Values are means of 6 heifers.

	Vitamin B_{12}		**Selenium**	
	nmol/kg fresh tissue		µmol/kg dry matter	
Treatment	91 days	280 days	91 days	280 days
Untreated	178[a]	110[a]	3.3[a]	2.7[a]
1 Co-Se pellet	246[ab]	200[b]	6.3[b]	4.4[a]
1 Co + 2 Se pellets	278[b]	322[c]	17.5[c]	8.3[b]

For each column, values with a different superscript differed at $P<0.05$.

CONCLUSIONS

Plasma

Vitamin B_{12} values in sheep are of diagnostic significance but such assays in cattle plasma appear problematic. Early detection of deficiency may require measurement of both plasma Vitamin B_{12} and metabolic indicators. Plasma MMA appears to be the preferred test for the early detection of marginal Vitamin B_{12} deficiency in ruminants. Reference ranges have yet to be established.

The recent development of a lactide/glycolide copolymer containing Vitamin B_{12} permits long term protection of young ruminants against Co deficiency as has been achieved with the intraruminal Co pellet in weaners. In many areas of Australia and New Zealand, livestock are at risk from both Co and Se deficiency. There may be an opportunity for commercial suppliers of supplements to 'value add' by combining the long-acting Vitamin B_{12} with barium selenate to produce a long acting product for young ruminants and to combine Co and Se in intraruminal high density pellets as has been achieved with the large boluses containing multiple trace nutrients.

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