

Cobalt concentrations in pasture species grown in several cattle grazing areas of Queensland

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Summary

Cobalt concentrations were determined in several pasture species grown in five cattle grazing areas of Queensland. Dry matter cobalt levels were within the range 0.01 to 0.35 $\mu\text{g/g}$ and were considerably higher in legumes than in grasses grown at the same location.

Seasonal changes in plant cobalt were variable and in some cases tended to coincide with changes in physiological maturity of the plant. Distribution of cobalt within some grass species and variability due to soil type differences within a grazing paddock were shown to be factors to consider when assessing the overall cobalt status of a pasture.

Pasture analyses indicated that beef cattle, which had recorded live weight gains following cobalt supplementation, had grazed pasture herbage containing 0.01 to 0.05 $\mu\text{g Co/g}$ dry matter.

INTRODUCTION

The essential role of cobalt in the nutrition of sheep, cattle and other animals is well known. Cobalt is an integral part of vitamin B12 and most workers now believe that cobalt deficiency in ruminants is essentially a deficiency of vitamin B12 (Marston 1952; Underwood 1962). Studies have shown that cattle and sheep grazing on cobalt deficient pastures for an extended period gradually lose appetite and weight, become anaemic and weak, and finally die (Underwood 1977). There is no clearly defined minimum concentration of cobalt required in pasture herbage for the maintenance and growth of ruminants. Most studies have indicated that a cobalt deficiency in cattle may occur when the feedstuffs contain less than 0.07 to 0.1 $\mu\text{g Co/g}$ dry matter (Young 1960; Kubota 1964; Underwood 1977) although others have suggested a critical level of 0.04 to 0.05 $\mu\text{g Co/g}$ (Hopkirk and Grimmett 1938; Smith and Loosli 1957; MacPherson, Moon and Voss 1973).

It is difficult to determine a more precise estimate of the minimum cobalt requirements of animals. Cobalt uptake in plants is influenced by variables such as availability of the cobalt in the soil upon which the plants have grown, species type, stage of maturity of the plant, climate and seasonal effects. Coupled with these is the usual problem of contamination associated with trace element analyses.

In Queensland, recent studies have shown positive responses in liveweight gains to cobalt therapy in cattle at 'Palm Range', a property near Bundaberg (Nicol and Smith 1981; Nicol, Smith, Dimmock, Green, Murphy and Barry 1983) and at properties near Mareeba (Holmes and Noble, pers. comm., 1978) and Tully (Teitzel and Holmes, pers. comm., 1981). Because of these responses to cobalt, and the limited amount of published data on cobalt levels in Queensland pasture (Bryan, Thorne and Andrew 1960; Winter, Siebert and Kuchel 1977) this study was undertaken. The aim was to measure the cobalt status of selected pastures and specific species, especially in areas where 'ill thrift' or cobalt deficiency in cattle was suspected.

The study was conducted in two stages. In 1979, an intensive pasture sampling programme was carried out at Palm Range to measure variability in cobalt levels between sampling points within one property. This within-paddock variability was used to assess the number of samples necessary to estimate plant cobalt to within some allowable error for a second, more extensive sampling programme in 1981.

This paper reports results of these studies and discusses some factors which affect the cobalt status of pastures. It also examines the use of these results for indicating cobalt deficiency in grazing animals.

MATERIALS AND METHODS

Intensive pasture sampling programme, 1979

The experimental site was the same 35 hectare paddock within Palm Range, a property 80 km north of Bundaberg, Queensland used by Nicol *et al.* (1983) for their animal studies (see Bundaberg, Table 1).

Table 1. Location, soil type and pasture species grown at each property

Property	Location	Soil classification	Pasture species
Bundaberg			
Palm Range	24°34'S 151°49'E	†Siliceous sand, ‡Uc 2.23	Green panic Siratro
Briarwood	24°36'S 151°48'E	Soloth, Dy 4.32	Green panic Siratro
Julatten (near Mareeba)			
Paddock 1	16°45'S 145°39'E	Xanthozem, Gn 3.71	<i>Panicum maximum</i> cv. Hamil, (Hamil grass)
Paddock 2	16°48'S 145°33'E	Krasnozem, Gn 3.11 (2)# Xanthozem, Gn 3.84 (1)	Hamil grass
King Ranch (near Tully)			
Paddock 1	18°10'S 145°45'E	Alluvial, Uf 1	<i>Panicum maximum</i> (common Guinea grass) <i>Centrosema pubescens</i> (Centro)
Paddock 2	18°15'S 145°47'E	Yellow earth, Um 4.23	Common Guinea grass Centro
Rockhampton			
Paddock 1	23°20'S 150°50'E	Grey clay, Ug 5.2	<i>Aristida</i> spp. <i>Eragrostis</i> spp.
Paddock 2	23°0'S 150°30'E	Brown clay, Ug 5.3	<i>Chloris gayana</i> (Rhodes grass) <i>Dichanthium sericeum</i> (Queensland blue grass)
Wandoan			
Flagstone	26°20'S 149°55'E	Grey clay, Ug 5.14	Green panic <i>Cenchrus ciliaris</i> (Buffel grass)
Shipfield	26°40'S 148°55'E	Grey clay, Ug 5.16	Green panic Rhodes grass

†Great soil groups (Stace, Hubble, Brewer, Northcote, Sleeman, Mulcahy and Hallsworth, 1968).

‡Northcote, Hubble, Isbell, Thompson and Bettenay (1975).

#Denotes number of points at which soil type identified.

The pasture was dominantly green panic (*Panicum maximum* var. *trichoglume* cv. Petrie) and Siratro (*Macroptilium atropurpureum* cv. Siratro) sown in October 1972. Superphosphate was applied annually since then at the rate of 125 kg/ha. Earlier pasture samples taken from this property contained cobalt levels of 0.20 to 0.75 µg/g which were considerably higher than previously reported Queensland data of 0.01 to 0.20 µg/g (Bryan *et al.* 1960; Winter *et al.* 1977) from areas where cobalt deficiency had been suspected or

had occurred. Contamination was suspected and the two most likely sources of this would be in the field (from dust or animal contamination) and during sample preparation. To minimise field contamination, exclusion cages were used and gloves worn while harvesting. To eliminate contamination from grinding, samples were handcut into small pieces with stainless steel scissors.

Ten exclusion cages (3 m×1 m) were placed at random over the paddock. Whole plant samples of green panic and Siratro were taken from these in February when the pasture was young and growing vigorously, in May when pastures were reaching late flowering to seed stage, and in September when growth was slow and plant material was old and matured.

Extensive sampling programme, 1981

Properties of paddocks from five locations were selected on the basis that cattle on them had either already demonstrated a response to cobalt therapy (Bundaberg, Mareeba and Tully) or were showing an ill thrift condition and generally retarded growth rate (Rockhampton and Wandoan). For each of these suspected cobalt deficient properties or paddocks, a neighbouring paired one was chosen where there was no evidence of cattle ill thrift. The Palm Range site near Bundaberg was that used in 1979. Table 1 shows the location, soil type and pasture species growing at each property.

Three pegs or points of reference were placed at random within each paddock. At each point a sample was taken of the two major pasture species present, except at Julatten where only one species was available. Each sample was a composite of 10 random cuts taken within a radius of 10 metres from the reference point. The whole plant was sampled by cutting 7 to 8 cm above ground with either stainless steel scissors or cleaned secateurs. In addition, diagnostic leaf samples (last fully expanded leaf blade) of Hamil and common guinea grasses were collected at Julatten and King Ranch respectively. Disposable vinyl gloves were worn at all times during harvesting and handling of samples, and exclusion cages used where possible. Pastures were sampled in February, May and September, except at Wandoan where sampling in May was not possible. At each experimental paddock, soil descriptions were carried out by Departmental soil surveyors to identify the soil type at each of the three sampling points.

Chemical and statistical analyses

Samples were oven dried at 80°C for 24 h and 1 g was taken for digestion in nitric/perchloric acid. After complexing with 1-nitroso-2-naphthol (Jago, Wilson and Lee 1971) and extracting into methyl isobutyl ketone, cobalt in the extract was measured on a Varian model 63 carbon rod analyser.

Analytical precision was determined by analysis of variance of the data obtained from three laboratory reference plant samples analysed in triplicate on each of three days. Analysis of variance was also used to obtain an estimate of the field sampling error with time effects removed at Palm Range in 1979, and to compare paddocks and time separately for each species at each location sampled in 1981. The paddock term in the analysis was tested against variability within paddocks. A paired *t*-test was used to examine differences between plant parts within each site at Julatten and King Ranch and between species at any one site.

To calculate the sample size required to give a mean with a 95% confidence interval of $\pm L$, the following formula was applied:

$$n = \frac{t^2 (\text{EMS})}{L^2} \quad (1)$$

where n =no. samples required
 t =value from student's t -tables for $n - 1$ degrees of freedom.
 EMS =error due to field sampling found at Palm Range in 1979
 L =maximum allowable error in estimate

RESULTS

Intensive sampling programme, 1979

Mean cobalt concentrations in green panic in February, May and September were 0.015, 0.016 and 0.029 $\mu\text{g/g}$ d.m. respectively while at the same times Siratro contained 0.036, 0.048 and 0.040 $\mu\text{g/g}$. For green panic the cobalt level in September (0.029 $\mu\text{g/g}$) was higher ($P < 0.05$) than in either February or May. The values for Siratro were higher ($P < 0.05$) than for green panic only in February and May.

The estimations of the within paddock sampling errors for green panic and Siratro were 0.51×10^{-4} and 0.11×10^{-3} respectively, Siratro being more variable between positions than green panic. A mean value was calculated from the above sampling errors and used in formula (1). An analysis of variance of the precision test data carried out in the laboratory gave a reproducibility standard deviation (Youden and Steiner 1975) of 0.008 $\mu\text{g/g}$. A relative confidence interval ($P = 0.05$) for a single estimate of cobalt content, calculated from this reproducibility s.d., was $\pm 17\%$ about a mean of 0.096 $\mu\text{g/g}$. Therefore 0.01 was chosen as the maximum allowable error in estimating a sample mean around 0.05 $\mu\text{g/g}$. Substituting this value for L in formula (1) gave a value for n of 4. A practical pasture sampling strategy of taking three bulk samples per paddock for analysis, each bulk sample being a composite of ten subsamples, was considered adequate based on the within paddock variance at Palm Range.

Extensive sampling programme, 1981

Location and site differences

Cobalt concentrations in pastures grown at different locations generally fell into two ranges (Table 2). Those grown at Julatten, King Ranch and the Palm Range site, Bundaberg, contained < 0.05 $\mu\text{g/g}$ while those at Wandoan, Rockhampton and the Briarwood site, Bundaberg contained > 0.1 $\mu\text{g/g}$.

Within some locations and species there were paired site differences. At Bundaberg, both green panic and Siratro from Palm Range contained lower ($P < 0.05$) levels of cobalt (up to ten times less) than did similar species from Briarwood. At Julatten, Cobalt in Hamil grass was lower ($P < 0.05$) in Paddock 1 (0.009 $\mu\text{g/g}$) than in Paddock 2 (0.032 $\mu\text{g/g}$). There were insufficient data to confirm differences in cobalt levels in green panic between Flagstone and Shipfield.

Species differences

Where legumes were a component of the pasture (Bundaberg and King Ranch) their concentrations of cobalt were higher ($P < 0.01$) than in grasses by a factor of two or more. Differences in cobalt concentrations among grass species at the same site (Rockhampton and Wandoan) were not confirmed (Table 2).

Seasonal differences

Seasonal changes in cobalt levels in the pastures were quite variable even for the same species within a location. At Palm Range, Siratro contained a higher ($P < 0.05$) cobalt concentration in September than in February and May whereas the same species grown at Briarwood was higher ($P < 0.05$) in May. At King Ranch Paddock 2, both common guinea grass and centro were higher ($P < 0.05$) in cobalt concentration in May and September than in February, while at Paddock 1, no seasonal differences in cobalt levels in the same

species were apparent. Hamil grass from Julatten Paddock 2 contained higher ($P < 0.05$) cobalt concentrations in September than in February and May. High variability in results at Rockhampton and insufficient data at Wandoan prevented any indication of seasonal differences at these locations.

Table 2. Cobalt concentrations in pasture species from five cattle grazing areas of Queensland

Locations and sites	Pasture species	Co ($\mu\text{g/g}$) Date of cut			Mean	l.s.d. (time effect) $P=0.05$
		Feb	May	Sep		
1 Bundaberg						
Palm Range	Green panic	0.013	0.015	0.019	0.016 a†	n.s.
	Siratro	0.027	0.032	0.041	0.033 b	0.009
Briarwood	Green panic	0.107	0.138	0.110	0.118 a	n.s.
	Siratro	0.225	0.363	0.207	0.265 b	0.122
l.s.d. (site effect) $P=0.05$					0.101 0.135	
2 Julatten						
Paddock 1	Hamil grass	0.008	0.009	0.009	0.009	n.s.
Paddock 2	Hamil grass	0.024	0.025	0.048	0.032	0.022
l.s.d. (site effect) $P=0.05$					0.021	
3 King Ranch						
Paddock 1	Common Guinea grass	0.020	0.013	0.025	0.019 a	n.s.
	Centro	0.036	0.032	0.045	0.038 b	n.s.
Paddock 2	Common Guinea grass	0.010	0.026	0.022	0.019 a	0.006
	Centro	0.028	0.045	0.039	0.037 b	0.011
l.s.d. (site effect) $P=0.05$					n.s. n.s.	
4 Rockhampton						
Paddock 1	Aristida	0.146	0.268	0.362	0.259 a	n.s.
	Eragrostis	0.295	0.344	0.152	0.264 a	n.s.
Paddock 2	Rhodes grass	0.072	0.197	0.161	0.143 a	n.s.
	Blue grass	0.072	0.143	0.116	0.110 a	n.s.
5 Wandoan						
Flagstone	Green panic	0.059	n.d.	0.120	0.09 a	n.s.
	Buffel grass	0.103	n.d.	0.111	0.107 a	n.s.
Shipfield	Green panic	0.073	n.d.	0.212	0.143 a	n.s.
	Rhodes grass	0.069	n.d.	0.167	0.118 a	n.s.
l.s.d. (site effect) $P=0.05$					n.s.	

†Within a site, species means followed by the same letter are not significantly different at $P=0.05$

n.d. no data

Plant part differences

Table 3 compares cobalt concentrations in whole plant and diagnostic leaf samples (youngest fully expanded leaf blade) of grass species grown at Julatten and King Ranch. At both King Ranch sites the mean yearly cobalt concentrations in whole plant samples of common guinea grass were higher ($P < 0.01$) than in the diagnostic leaf material. This pattern of cobalt distribution occurred at each sampling time. Similar results were obtained for Hamil grass at the Paddock 2 Julatten site in February and May. Although the difference in the mean yearly cobalt concentration between plant parts was not significant, the difference in the mean for the February and May samplings was sufficient to attain the 5% level of significance. In September, when fresh regrowth was sampled following a fire,

and the whole plant was essentially at the same physiological age as the diagnostic leaf, no true comparison between plant parts could be made. At Julatten Paddock 1, where cobalt levels were very low, no difference between plant parts was detected.

Effect of non-uniformity of soil type within a site

Soil descriptions confirmed the uniformity in soil type between pasture sampling points at all sites except Julatten Paddock 2. Here, Hamil grass was grown on a krasnozem soil at two points and on a xanthozem at the third (Table 1). Also, the mean yearly cobalt concentrations in Hamil grass grown on krasnozem soil were 0.042 $\mu\text{g/g}$ (whole plant) and 0.036 $\mu\text{g/g}$ (diagnostic leaf). The corresponding cobalt levels in the same species grown on the xanthozem were 0.015 $\mu\text{g/g}$ and 0.014 $\mu\text{g/g}$ respectively. This pattern of higher plant cobalt uptake from the krasnozem soil relative to the xanthozem occurred on each sampling occasion.

Table 3. Cobalt concentrations in leaf blade and whole plant samples of Hamil and common Guinea grasses

Location	Plant part and species	Co ($\mu\text{g/g}$) Date of cut			Mean	
		Feb	May	Sep		
Julatten Paddock 1	Hamil grass					
	Whole plant	0.008	0.009	0.009	0.009 <i>a</i> *	
	Leaf	0.008	0.008	0.012	0.009 <i>a</i>	
Paddock 2	Whole plant	0.024	0.025	0.048†	0.032 <i>a</i>	
	Leaf	0.013	0.017	0.056†	0.028 <i>a</i>	
King Ranch	Common					
	Guinea					
	Paddock 1	Whole plant	0.020	0.013	0.025	0.019 <i>a</i>
		Leaf	0.011	0.008	0.012	0.010 <i>b</i>
	Paddock 2	Whole plant	0.012	0.026	0.022	0.019 <i>a</i>
		Leaf	0.006	0.015	0.011	0.011 <i>b</i>

*Within a paddock, means followed by the same letter are not significantly different at $P=0.05$.

†Fresh regrowth sampled shortly after fire burnt out paddock.

DISCUSSION

The range of cobalt levels found in these Queensland pastures agrees with the general finding that most pasture herbage contains 0.01 to 0.35 $\mu\text{g Co/g}$ (Young 1979).

The variance due to sampling errors in a paddock can differ between species as illustrated at Palm Range in 1979. Such estimates of variability are combinations of (1) true within-paddock variability, (2) contamination error and (3) analytical error. Since a large proportion of variability was due mainly to one or two high results in each group, the most likely causes of this variability is sporadic contamination error. Since the difference between adequate and deficient pasture cobalt levels for animals is small in analytical terms, a careful approach to both sampling and analysis is essential. Earlier work indicated that the major source of cobalt contamination was from grinding processes. However, if possible, exclusion cages should be used to prevent chance contamination by stock prior to sampling.

Evidence of seasonal variation in pasture cobalt was difficult to assess. True seasonal effects could be gauged only by comparing cobalt concentrations in Hamil and common guinea grass leaves of constant physiological age sampled throughout the year at Julatten and King Ranch respectively. From these results (Table 3) no seasonal trend was evident. However, significant seasonal differences were found in cobalt concentrations of whole

plants sampled from a number of locations (Table 2), but in some cases seasonal change was confounded with change in physiological maturity of the pasture. This is evident at Julatten Paddock 2 where the higher cobalt concentration found in Hamil grass in September is most likely due to the younger plant material sampled rather than true seasonal effects.

Significantly higher cobalt concentrations in legumes than in grasses found from this study have also been noted by others (Latteur 1962; Fleming 1965; Ssekaalo 1972). This suggests that an increase in legume component of low cobalt pastures at Palm Range and King Ranch may help alleviate cobalt deficiency problems occurring there.

Except when sampling fresh growth after fire (Julatten Paddock 2, September harvest, Table 3) or when cobalt levels were very low throughout the year (Julatten Paddock 1, Table 3), cobalt concentrations in whole grass samples were higher than in leaf blade samples. This pattern of cobalt distribution for Hamil and common guinea grasses is similar to that for other grasses studied in water culture by Handreck and Riceman (1969). They found that cobalt accumulated in the youngest leaves and that maximum concentration in fully expanded leaves occurred in the leaf sheath. The implication of non-uniform plant cobalt distribution is that grazing habit could influence cobalt intake by the animal.

Soil type has a major influence over plant cobalt levels (Gardiner 1977). In this review Gardiner showed that highly weathered soils and soils derived from acid igneous rocks tend to be low in cobalt while soils of basaltic origin are generally cobalt adequate. Similarly Kubota (1968) reported a high positive correlation between soil cobalt and clay content. Soils described in this study (Table 1) confirm this pattern, with cobalt adequate pastures being grown on basaltic clays at Rockhampton and Wandoan while cobalt deficient pastures are associated with the weathered sand at Palm Range and the krasnozem, xanthozem and alluvials derived from granite at Julatten and King Ranch. The higher clay content in the soloth at Briarwood could explain the higher plant cobalt levels found there compared to those at Palm Range.

Difference in plant cobalt between sampling points in Julatten Paddock 2, appears to be the result of different soil types, the krasnozem being derived from basic volcanic parent material and the xanthozem from metamorphosed Hodgkinson formation material, dominantly sandstones and siltstones (Department of National Development 1962). This occurrence of different soil types within a grazing area further emphasises the need for proper sampling procedures, hence proportional distribution of soil types should be taken into account when assessing the overall cobalt status of pastures in an area.

The value of plant cobalt analysis as an index of potential cobalt deficiency in ruminants can only be assessed in conjunction with animal experiments. Animal response to cobalt supplementation has been obtained at Palm Range and at Paddock 1 of both Julatten and King Ranch. For these three sites, cobalt content in pastures was consistently less than 0.05 µg/g dry matter throughout the year. On the other hand, there was no apparent ill health in animals grazing Paddock 2 at either Julatten or King Ranch even though plant cobalt analyses suggest that these pastures could be cobalt deficient. In fact, at Julatten, animals kept on Paddock 1 wasted away and finally died while affected animals shifted to Paddock 2 quickly recovered (Holmes and Noble, pers. comm., 1978). The yearly average for Hamil grass in Paddock 1 was 0.01 µg Co/g (range 0.007 to 0.01) while that for Paddock 2 was 0.03 µg Co/g (range 0.01 to 0.07), suggesting that cattle can be maintained in apparently good health on dietary cobalt levels averaging 0.03 to 0.04 µg/g, at least in the short term. However, this could also depend on the animal's ability to select a diet of higher quality if such a diet was available to it as in Julatten Paddock 2. Furthermore, the relatively large range of values found in Paddock 2 includes both critical levels of cobalt requirements for cattle, as suggested by other workers, and emphasises the problem of using plant cobalt analysis for assessing cobalt deficiencies in ruminants.

Pasture cobalt levels at Rockhampton, Wandoan and Briarwood (near Bundaberg) indicate that cobalt deficiency in cattle is unlikely to be the cause of any ill thrift problems that might occur at these locations.

Results reported here indicate the range of cobalt levels in Queensland pastures. Non-uniformity of soil types within a grazing area, species, plant cobalt distribution and stage of growth are identified as factors affecting cobalt status of pastures. Where animal responses to a cobalt supplement have been measured, pasture analysis has confirmed this dietary deficiency. Similarly such analyses were useful in eliminating cobalt as a cause of animal ill thrift in cobalt adequate areas. However, low levels found in selected pasture species in some areas may not be sufficient in themselves to justify a definite diagnosis of cobalt deficiency.

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