INVESTIGATION OF VITAMIN $B_{12}$
DEFICIENCY IN RUMINANTS

A Thesis

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by

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SUMMARY

Early detection of vitamin B₁₂ deficiency in livestock is of economic importance especially in cases where no overt signs are readily apparent. Metabolic indicators of vitamin B₁₂ deficiency were examined in animals in which the deficiency was induced with either nitrous oxide (N₂O) or by feeding diets of low cobalt content.

Short term N₂O experiments involved exposing sheep and cattle to N₂O over 5 hour periods. This short term exposure readily inhibited the activity of the vitamin B₁₂ dependent enzyme methionine synthetase, while there was only slight inhibition of methylmalonyl CoA mutase activity in liver. Metabolites of the methionine synthetase pathway were affected by N₂O exposure as indicated by increases in plasma homocysteine and urinary formiminoglutamic acid (FIGLU), and decreases in plasma free methionine concentrations. Plasma and urine methylmalonic acid (MMA) concentrations were unaffected by N₂O exposure suggesting little disturbance of the methylmalonyl CoA mutase pathway.

A longer term N₂O experiment (6 weeks) was undertaken with cattle and rats. Cattle and rats were exposed to N₂O for one hour per day for four weeks and then twice per day (twelve hours apart) for a further two weeks. In cattle, total and holo methionine synthetase activities in liver were almost completely suppressed at week 6, whereas in rat liver total and holo methionine synthetase activities were partially suppressed. There was no inhibition of methylmalonyl CoA mutase activity in liver of cattle but in rat liver the holo enzyme activity was halved. Metabolites of the methionine synthetase pathway, plasma homocysteine and urine FIGLU concentrations were significantly increased in cattle. In rats
a significant decrease in liver vitamin B$_{12}$ concentration was found. No change in plasma MMA or succinic acid concentrations was detected in either species.

Vitamin B$_{12}$ deficiency induced with N$_2$O appears to affect only the methionine synthetase pathway in cattle and sheep resulting in increases in plasma homocysteine and urine FIGLU concentrations. In the rat, both the liver total and holo methionine synthetase and holo methylmalonyl CoA mutase activities were inactivated to a similar degree and liver vitamin B$_{12}$ concentrations reduced, no metabolites of the methionine synthetase pathway were measured, and there was no change observed in plasma MMA concentrations despite inactivation of this pathway.

With regard to feeding diets of low cobalt content, two experiments with young cattle and one with young sheep were conducted over 6 to 9 months. The rations offered were of low cobalt content and for each experiment the animals were allocated to one of three treatment groups, viz: untreated, paired with untreated group and supplemented with cobalt, and ad-lib fed and supplemented with cobalt.

The untreated cattle in both experiments were of low vitamin B$_{12}$ status (liver vitamin B$_{12}$ < 400 nmol/kg) however appetite and liveweights were unaffected. The activity of the vitamin B$_{12}$ dependent enzymes in liver were not suppressed by the low cobalt diet but there were significant decreases in vitamin B$_{12}$ and increases in succinic acid concentrations in rumen fluid. Significant reductions in plasma $\alpha$-leucine concentrations mirrored those of liver vitamin B$_{12}$. 
In sheep of marginal vitamin B\textsubscript{12} status (liver vitamin B\textsubscript{12} < 200 nmol/kg), vitamin B\textsubscript{12} dependent enzymes in liver were not altered by cobalt treatment. The earliest changes associated with onset of vitamin B\textsubscript{12} depletion were plasma \(\alpha\)-leucine and MMA, and rumen fluid succinic acid and vitamin B\textsubscript{12} concentrations. Other metabolites which changed prior to liver vitamin B\textsubscript{12} concentration were urinary MMA and FIGLU, and plasma free methionine and serum holo transcobalamin II concentrations.

Vitamin B\textsubscript{12} dependent enzymes in liver of ruminants appear to be affected only at a late stage in nutritional vitamin B\textsubscript{12} deficiency. Despite this, changes in concentrations of metabolites of these pathways occurred earlier. Plasma MMA and plasma \(\alpha\)-leucine concentrations were affected early in nutritional vitamin B\textsubscript{12} deficiency and are therefore considered to be indicators of marginal vitamin B\textsubscript{12} inadequacy in sheep. Plasma \(\alpha\)-leucine was also decreased in marginal cobalt deficiency in cattle and this is the first report of changes in this metabolite in cobalt deficiency in ruminants. Plasma holo transcobalamin II also was decreased in nutritional cobalt deficiency in sheep and in N\textsubscript{2}O treated cattle. This is a new indicator of vitamin B\textsubscript{12} deficiency in ruminants which is worthwhile pursuing as a diagnostic aid. The use of a radio-isotope dilution assay (Diagnostic Products Corporation (DPC) no-boil, extracted at 100°C) for measurement of vitamin B\textsubscript{12} in bovine and ovine blood plasma has been validated, being shown to totally denature the endogenous vitamin B\textsubscript{12} binders in plasma.