PROTECTION OF CATTLE FROM FLUOROACETATE POISONING BY GENETICALLY MODIFIED RUMINAL BACTERIA

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Fluoroacetate poisoning in cattle occurs as a result of their consumption of Australian plants like acacia (*Acacia georginae)* and heartleaf (*Gastrolobium grandiflorum)*, where this compound occurs at up to 5 g/kg DM. The LD_{50} of this compound in ruminants is about 0.3 mg/kg body weight. A previous study showed that sheep inoculated orally with *Butyrivibrio fibrisolvens* microbes, transformed with fluoroacetate dehalogenase plasmid (pBHf), provided markedly reduced toxic symptoms when challenged with the poison (Gregg *et al.* 1998).

Twenty Angus steers (10 control and 10 test) were used to test whether pBHf-bearing *B. fibrisolvens* strains, when inoculated orally, were able to protect against fluoroacetate. Under appropriate containment conditions, uninoculated control animals were fed 3 equal doses of 0.11 mg/kg body weight of sodium monofluoroacetate, at 0, 3, and 6 h, and their behaviour and heart rates monitored. Five animals showed acute symptoms of toxicity, were judged to be intoxicated, and were euthanased between 11-15.5 h. The remaining controls survived the challenge, albeit with reduced rumination and mobility.

Test animals were inoculated with 50 mL of each of 7 *B. fibrisolvens* strains containing the pBHf plasmid. Establishment of the recombinant bacteria to 10^4-10^7/mL was confirmed by PCR of rumen fluids collected 7, 12 and 15 days post-inoculation. When challenged with the same regime of sodium monofluoroacetate as the controls, none of the test animals showed acute symptoms of toxicity. Although all animals showed some signs of reduction in activity, they continued to ruminate at normal levels for the 15 h of physiological and behavioural monitoring. Post-mortem PCR of rumen fluid dilutions showed that pBHf transformed *B. fibrisolvens* was stably colonised from 10^5-10^7 cells/mL (Table 1) 20 d post-inoculation. Post-mortem rumen fluid samples that were serially diluted 10-fold to 10^{-6}/mL, and enriched overnight in anaerobic media, all returned a positive PCR for the presence of bacteria containing the dehalogenase gene. This suggests that the rumen fluid in all 10 test animals contained $\geq 10^6$/mL of fluoroacetate degrading bacteria at the time of slaughter.

Table 1. Post-inoculation *B. fibrisolvens* cell densities (cells/ml) in rumen fluid dilutions.

	1	2	3	4	5	6	7	8	9	10
Bacteria 7 d (cells/mL)	10^5	10^5	10^4	10^5	10^5	10^6	10^5	10^4	10^4	10^5
Bacteria 15 d	10^5	10^5	10^5	10^5	10^5	10^5	10^5	10^5	10^5	10^5
Bacteria 20 d	10^7	10^5	10^6	10^6	10^5	10^6	10^6	10^6	10^5	10^5

In this study, the dose of fluoroacetate was calculated as 10% above the LD_{50} for cattle, and the acute symptoms of fatal toxicity in 50% of the control animals matched the predicted outcome. The lack of acute symptoms in all inoculated test cattle represented a significant reduction in toxicity. It is clear from this study that modified rumen bacteria are capable of reducing toxic effects of fluoroacetate in cattle. Field use of the technology will depend on approval for release by the Office of the Gene Technology Regulator. If released for use, this technology could benefit animal producers across the Georgina river basin, Queensland heartleaf regions, and parts of northern and Western Australia where economic losses from fluoroacetate poisoning are significant.

GREGG, K., HAMDORF, B.J., HENDERSON, K.P., KOPECNY, J. and WONG, C. (1998). *Appl. Environ. Microbiol*. **64**, 3496-3498.

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