BRIEF COMMUNICATION: Reducing the risk of photosensitisation in dairy cattle undergoing a facial eczema challenge

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Introduction

Facial eczema (FE) is primarily a liver disease of ruminants that is caused by the ingestion of the toxin sporidesmin which is produced by spores of the fungus, *Pithomyces chartarum*. The liver and bile-duct injury may be monitored with a non-specific indicator, gamma-glutamyltransferase (GGT; Morris et al. (1990)). Dairy cattle may be assessed for their tolerance/susceptibility to sporidesmin by measuring their GGT activities in blood, about three weeks after a sporidesmin challenge. Under these conditions, $\log_e$GGT is heritable (0.34 ± 0.02), according to Cullen et al. (2011).

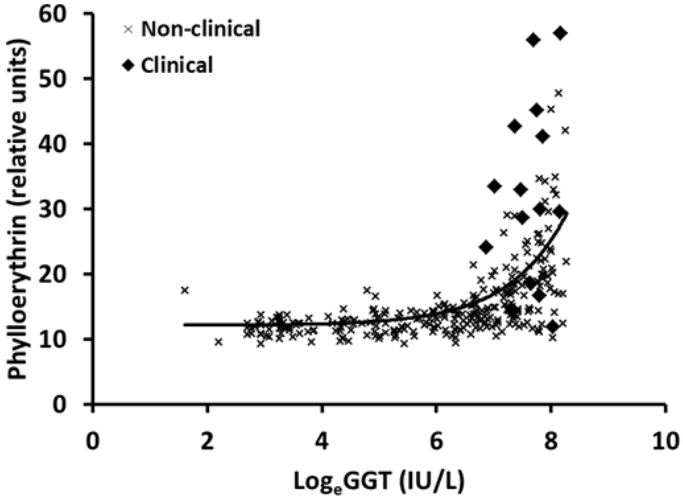
Also, the concentration of blood phytoporphyrin, a photodynamic break-down product of chlorophyll *a*, can be measured. Phytoporphyrin is formed during microbial metabolism of green forages in the rumen (Waghorn et al. 2002) and assimilated phytoporphyrin is excreted via the bile ducts to the intestine. It is suspected that the blockage of bile ducts after sporidesmin exposure leads to leakage of phytoporphyrin into the systemic blood-stream. Phytoporphyrin in skin capillaries is activated on exposure to sunlight, leading to secondary photosensitisation in cattle with light- or white-coloured skin and causing the skin lesions associated with FE.

In 2010, AgResearch was approached by a Bay of Plenty dairy-farming couple, with access to DairyNZ funding, to explore new avenues for identifying sporidesmin-tolerant bulls.

CRV Ambreed, a New Zealand herd-improvement company, collaborated in a FE performance test on young bulls selected for their NZ progeny-test programme. Animal-breeding companies have been reluctant to performance-test their bulls for FE because of adverse reaction to the challenge and possible delay of onset of puberty which would limit a bull's ability to produce semen as a yearling for progeny-test purposes.

High sporidesmin dosages would be expected to cause liver and bile duct injury in dairy cattle. GGT activity was measured in blood samples from 15,000 dairy cattle (mainly cows) exposed to a natural sporidesmin challenge in 2004-11 (Cullen et al. 2011); some of these samples were also analysed for phytoporphyrin.. It was found that no animal with a GGT activity below ~600 iu/L (i.e., $\log_e$GGT= 6.40) exhibited clinical signs of FE and, overall, there was only a weak relationship between $\log_e$GGT and phytoporphyrin concentration (Figure 1). It is not clear whether removing chlorophyll (green pasture) from the diet during a sporidesmin challenge, and thus reducing phytoporphyrin produced, will prevent secondary photosensitisation.

Figure 1. Relationship between logeGGT enzyme activity and plasma phytoporphyrin (relative fluorescence intensity) in cattle from a herd unintentionally exposed to sporidesmin. Log_eGGT values of 6.4 correspond with GGT activities of about 600 iu/L.



The aim of this work is to develop a modified sporidesmin challenge protocol that can be used to identify tolerant bulls without compromising growth and without inducing clinical FE in the bulls tested.

Materials and methods

Animal Management

Bulls were continuously housed in pens (in groups of 5 – 7 bulls per pen) on wood shavings without access to fresh pasture and were fed a diet of pasture silage and concentrate. The housing had translucent (UV permeable) plastic roofing over approximately half of each pen.

Trial design

The trial was carried out with the approval of the AgResearch Ruakura Animal Ethics Committee: Approval Nos. 12230 and 12602.

Cullen et al. (2013) described the initial pilot, trial and subsequent challenges over the next two autumns of a proportion of the latest intake of young bulls. In brief, the pilot trial in late-2010, using 16-month bulls, was designed to test the new protocol and establish the sporidesmin dose rate. Larger-scale testing of ~eight-month-old bulls commenced in autumn 2011. Candidates for testing were chosen mainly on the basis of parent-average breeding values for FE. The dose-rate in the second year (2012) was reduced by 10% (Table 1) to reduce the risk to animals from rare families.

Serum GGT activities were recorded prior to dosing (dosing day defined as Day0) to exclude any animals already showing liver damage. At 14 and 21 days post-sporidesmin dosing (Day14 and Day21), blood samples were collected to assess GGT activity responses. The serum samples of blood taken at Day21 from the bulls in 2012 were stored at -20°C and these were assayed for phytoporphyrin concentration.

Serum phytoporphyrin assay

Serum phytoporphyrin concentrations were measured by fluorescence intensity (Campbell et al. 2010) at 420 nm excitation wavelength, 10 nm bandwidth and 650 nm emission wavelength, in a BMG FLUORstar OPTIMA fluorescence plate reader. A series of phytoporphyrin standard solutions was made from a stock solution of phytoporphyrin (Porphyrin Products (now Frontier Scientific Inc.), Logan UT, USA) dissolved in dimethylsulphoxide (Sigma, St Louis, USA) to a concentration of 0.2 mM. The stock was stored frozen in aliquots at -20ºC. On the day of assay, the standards were prepared by mixing varying amounts of thawed phytoporphyrin stock solution with serum to final concentrations ranging from 0-48 µM. The sera for the phytoporphyrin standards were pooled from four individual animals determined to be phytoporphyrin-free. All samples and standards (200 µL) were analysed in duplicate in black-sided microtitre plates. The mean of 20 readings was obtained for each replicate sample and standard solutions and the phytoporphyrin concentration for the samples was calculated from the standard curve after deducting the reading of the phytoporphyrin-negative control serum.

Results and discussion

Results in terms of the number of bulls dosed, dose rates and distribution of GGT activities for all trials are shown in Table 1. In the pilot trial (Cullen et al. 2013), the first dose rate of 0.25 mg sporidesmin/kg live weight resulted in only 5/11 bulls showing a GGT response; 5 of the remaining 6 were subsequently re-dosed at 0.30 mg/kg and 3 of these also had an elevated GGT. In total, after two rounds of dosing, 2 bulls of the 11 showed no response to the sporidesmin doses. In 2011, 45 calves were dosed once with sporidesmin at a rate of 0.30 mg/kg live weight. In the following year (2012), 50 young bulls were challenged once with a dose at 0.27 mg/kg. A proportion of animals with GGT levels greater than 600 iu/L may be expected to show secondary photosensitisation.

Table 1. Summary of the three facial-eczema trials conducted on bulls in 2010-12; the pilot trial conducted in 2010 to establish a sporidesmin dose-rate sufficient to elevate GGT in a high proportion of animals, and the subsequent sporidesmin-dosing of some bulls from the latest intakes of bull calves. Oral dose rate (as a suspension in water) was expressed in mg sporidesmin per kg live weight of animal. GGT is gamma-glutamyltransferase.

Trial & Year		Age of bull (months)	Sporidesmin dose rate	No. dosed	No. with GGT >40	No. with GGT >600
'Pilot trial'	2010a	16	0.25	11	5	1
	2010b	17	0.30	5	3	0
Year 1	2011	8	0.30	45	38	22
Year 2	2012	8	0.27	50	43	18

None of the animals in any of the years showed any skin lesions or any of the early behavioural signs of photosensitivity such as head-shaking, tail-switching or irritability during handling at blood sampling. The lack of FE clinical signs were hypothesised to be due to a lack of phytoporphyrin in the blood-stream as animals were exposed to sunlight which is the other contributing factor in the development of clinical signs.

All 50 animals challenged in 2012 had levels of phytoporphyrin in serum <0.4 µM. The low serum phytoporphyrin concentrations support the revised protocol as being effective in eliminating cases of clinical FE, and a potential animal management tool during an FE outbreak.

Only relative units for phytoporphyrin in cattle were previously reported (Morris et al. 2009) because there were no standards available at the time. However, the concentrations recorded for sheep are in agreement with those of Campbell et al. (2010). Phytoporphyrin levels in sheep with GGT >450 iu/L range up to ~3.8 µM in both studies, while those in cows with clinical FE range between 0.4 and 1.8 µM (Campbell et al. 2010). In the work reported here, there was a tendency for the phytoporphyrin concentrations to increase with increasing GGT, especially when GGT levels were >600 iu/L, indicating there may still be residual chlorophyll in the silage.

Of the 95 young bulls challenged with sporidesmin, 40 had GGT >600 iu/L, the level above which we would expect to observe clinical FE in some of these animals. The absence of any signs of photosensitivity shows that the revised protocol is effective at preventing clinical FE in these valuable young bulls.

Acknowledgements

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