Assessing a research project with reference to the big picture

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Background

New Zealand has a large pastoral farming industry—5 million dairy cows, about 5 million beef cattle and 35 million sheep. The industry relies on predominantly ryegrass pasture.

Unfortunately, a saprophytic fungus (*Pithomyces chartarum*) has a predisposition to infect ryegrass. The fungus grows in the dead litter at the base of the pasture, but can also grow on the green leaves. Although Pithomyces species are also found in Europe, North and South America, South Africa and Australia, the prevalent New Zealand strains differ from those in the other countries in that 95% of them are toxigenic—so this is essentially a New Zealand problem.

A toxin (sporidesmin) that is produced by the spores is the causative agent of an animal disease called facial eczema (FE). The fungus produces spores when grass minimum temperatures are above 12°C for two or three nights and humidity is high. Warm, moist conditions on dry pastures after rain or heavy dews increase FE risk. Autumn is the highest risk period.

Affected animals have abnormal, hard, mis-shapen livers and jaundiced fat. The toxin from ingested spores damages the liver. The liver damage causes the release of two enzymes (gamma-glutamyl transferase or GGT and glutamate dehydrogenase or GDH), which when present in a blood sample are indicative of liver damage, but not always diagnostic for the onset of clinical FE. Badly damaged liver tissue will not regenerate. Chronic wasting and/or death may occur at the time of damage or months later when the animal is under stress.

The damaged liver cannot eliminate phylloerythrin—a chlorophyll breakdown product—and this circulates in the blood, releasing energy when activated by light and making the skin photosensitive. A photosensitive reaction similar to severe sunburn is the indicator of clinical FE. Early exposure to low levels of spores sensitises animals so that they get FE at lower spore intakes than animals not previously exposed. FE damage can occur from long-term intake of low numbers of spores, short-term intake of high numbers or a combination of both. Clinical FE appears 10–20 days after ingestion of the spores. By the time 3% of a group of animals are showing clinical signs, up to 95% can be sub-clinically affected.

The first signs of FE in sheep are drooping ears and swollen eyes. Affected animals shake their heads and rub their eyes on fence posts and gates, which causes sores and bleeding. The animals are desperate to find shade and are loathe to come out of the shade to eat or drink. The lesions are often attacked by blowflies. Badly affected sheep stop eating and often die very quickly. If the animals are detected soon enough, moved indoors into shade and fed hay, the skin damage can be repaired.

In cattle, the first signs are reddening and flaking skin on hairless skin and white skin areas (along the back, under the front legs, the udder and teats and around the eyes, ears, and nose). The teats can be so severely affected that cows cannot be milked. Animals are restless and are desperate to find shade. They try to nibble the affected areas, which are itchy—particularly when wet from rain. The flaking skin sloughs off (often in great lumps) and the raw flesh left beneath can become infected and sore.

About 70% of dairy cows, 60% of beef animals and 40% of sheep in New Zealand are farmed in FE-risk areas. As there is no cure for FE, prevention is the best option. The basis of prevention treatment is administration of zinc salts, but excess zinc is toxic and over a long FE season, animals often start to show the effects of too much zinc.

The NAEAC Code of recommendations and minimum standards for the welfare of sheep specifically refers to FE in Item 6.6 and there is a legal obligation to treat animals with clinical FE. Severe cases can be so bad that the animals must be euthanased.

Issues for the AEC to consider

Selecting for genetic resistance or tolerance is an acceptable option. FE tolerance has a relatively high heritability. Currently, rams are dosed with sporidesmin and their GGT response is measured from a blood sample, as an assessment of tolerance (or lack of tolerance). By selecting rams with low GGT levels following the challenge, tolerance can be increased in their offspring. But as tolerance increases in the rams following selection, the dose level required to elicit a response must also increase.

FE is most prevalent in the North Island and the northern parts of the South Island; it has never been reported in Southland. The AEC was presented with a protocol to generate a phenotyped sheep resource for the discovery of causative genes that affect FE resistance. By identifying such genes it is possible to select for tolerance based on non-invasive DNA tests, as opposed to having to artificially challenge animals with the toxin to measure their response. For the study, animals were to be transported from an FE endemic area (in Hawke's Bay) to Southland, where there is no natural FE challenge to interfere with the experimental protocol.

This caused a dilemma for the AEC. Animals were to be taken to a location where they were not at risk of FE, and then dosed with the causative toxin, which negatively impacts on animal welfare. There were sound scientific reasons for this:

- there was no natural challenge to interfere with study;
- in an endemic area it is impossible to control the amount of toxin animals are exposed to;
- in endemic areas, the prophylactic zinc treatments routinely administered to minimise risk would interfere with the study;
- the single dose of toxin given in the study would be much less invasive than the cumulative effects of successive unmoderated natural doses in a bad FE year.

Two breeds were chosen for their divergent reactions to the effects of the toxin. The Finnish Landrace is an FE-resistant breed and the Texel has natural susceptibility. Gene discovery is undertaken by crossing widely divergent phenotypes, and comparing the phenotype segregation to the DNA of the resulting family lines. By challenging the offspring with a fixed dose rate of sporidesmin, and measuring the resultant GGT and GDH levels, each animal's resistance or susceptibility to the toxin can be measured.

The AEC had obvious animal welfare concerns, and the conditions of the project were rigorously negotiated with the applicant:

Over 3 years, half-sibling family lines totalling 2000 individuals were to be challenged with sporidesmin. The dose rate was lower than the lowest commercial ram test dose, and was optimised for the two breeds used.

GGT and GDH levels were monitored weekly.

The work was undertaken when feed supply was good. This enabled growth rate to be used as a secondary measure of animal health status.

The grazing area included trees for shade and a shed was constructed to house photosensitive animals from sunlight.

The work was carried out when normal day-to-day farm management requirements were low, which allowed the personnel to commit to the animal welfare aspects of the study.

Clinical outcomes

During the course of the study, 60% of the animals recorded GGT levels which ranged from 71 to 1185. The accepted cut-off for sub-clinical FE is <70. All animals with elevated GGT were closely monitored. Subsequently, 15% became clinically affected, and were moved indoors to avoid sunlight. They were let outside for exercise and grazing during evening and night when the risk of exposure to sunlight was minimal. Unfortunately, 0.5% of the animals died. The majority of deaths were the result of euthanasias required because of ill-thrift of the animals, not because they were suffering.

Science outcomes

There is now a very extensive sheep genetic resource with accurate pedigrees and standardised phenotypes. This will be further augmented by sampling animals from the industry that have been exposed to natural FE challenge.

Selection lines of animals bred over the last 33 years are still diverging in their resistance/susceptibility levels, indicating that FE resistance is a multi-genic trait. New, powerful DNA analysis technologies will enable the investigators to scan the whole sheep genome to identify genes that are influencing resistance to FE. The technology is SNP chips (Single Nucleotide Polymorphisms). Once the SNPs are identified, a simple one-off blood DNA test will be used to select resistant animals with no need to challenge them with sporidesmin.

Animal welfare outcomes

There was undeniably an ethical cost to some of the animals in the study. Importantly though, this project addressed each of the Three Rs—not only from an animal-in-research point of view, but in the wider context of the farming industry as well.

By undertaking the project in an area where there was no natural challenge, the lowest dose necessary to measure a phenotype was administered—Refinement.

Using breeds with widely diverging natural phenotypes minimised the number of animals used to provide a useful resource—Reduction.

When the DNA that confers resistance (or susceptibility) is identified, detecting the presence or absence of these genes will be done from a simple one-off blood test; thus eliminating the need for performance testing by dosing sentient animals with a known toxin—Replacement.

A 4th R—Rationale—was also addressed. The value of the DNA and animal resources generated by this project cannot be overestimated. The most important benefit from the study is the potential to develop a simple diagnostic tool that will eliminate the need for artificially testing farm animals, and at the same time improve the welfare of millions of sheep and cattle every year under natural field conditions.