# Management of Facial Eczema

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# Abstract

Facial eczema is a hepatogenous photosensitisation of ruminants caused by sporidesmin produced by the saprophytic fungus *Pithomyces chartarum*. It is of large concern to the dairy industry, both with its effects on production and the significant animal welfare implications of affected stock.

In 2011 DairyNZ and Sustainable Farming Fund invested in research initially aimed at trying to get a better understanding behind the natural spore count variability in paddocks, between paddocks and between farms and to try and find alternative ways of managing facial eczema without zinc. After this research was completed in 2013 it was deemed necessary to try and understand to what extent management of facial eczema was breaking down and possible reasons for these breakdowns. The overall aim of this research was to try and help farmers improve their management of this disease and reduce incidence of facial eczema.

A study comparing the spore counts from paddocks containing varying quantities of herbs, clovers and tall fescue showed that the addition of chicory, plantain, lucerne and white clover into a ryegrass pasture did not provide any reduction in spore counts. Tall fescue paddocks showed lower spore counts over time than pure swards of ryegrass.

A study comparing the application of lime and nitrogen in comparison to control paddocks showed that application of lime before the risk period for facial eczema (in November), application of lime after a spore count rise, (in March) or urea application (in December) did not affect the number of spores produced by *Pithomyces chartarum*.

A study investigating the variability of spore counts within farm, paddock, grass sample and water aliquot showed that if spore counts are to be used for monitoring purposes to identify when to start and finish facial eczema(FE) prevention programmes, at least three aliquots per wash water should be selected.

Finally, a study looking at the different types of management of FE used and their effectiveness highlighted that FE management on dairy farms in New Zealand could be substantially improved;

principally through farmers getting more information on the success of their FE management programs and responding when tests show that FE management is not effective.

# Acknowledgements

It is not until you reach the point of writing the acknowledgements of a thesis that you truly appreciate the number of people that contribute to the completion of such a body of work.

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This is has truly been a wonderful experience and I hope it makes a difference within the dairy industry.

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# Chapter 1

# Introduction

Facial eczema (FE), or pithomycotoxicosis, is a disease of ruminants and camelids caused by the saprophytic fungus *Pithomyces chartarum* (Thornton 1959). FE has occurred in New Zealand since at least 1897 when reference was first made to it in a report by the New Zealand Department of Agriculture (Cunningham *et al.* 1942), although the cause was not discovered until over 60 years later (Brook 1969). FE occurs in late summer and autumn mainly in the North Island and the top end of the South Island (Andrew 1957) However it has occurred in the South Island as far south as Geraldine. The disease is most commonly seen seasonally in New Zealand but has been reported in many countries where livestock graze warm temperate pastures, including Australia, South Africa, France, Spain, the Netherlands, Portugal, Uruguay, Argentine and Brazil (Collin and Towers 1995a; Collin *et al.* 1998)

The term 'facial eczema' like many old disease terms, such as milk fever, does not truly describe the disease. Effects on the face and head are only a small part of the clinical picture, especially in cattle (Clare 1952; Dodd 1959), while 'eczema' is generally recognised as broadly applying to a range of persistent skin conditions from dermatitis to rashes. However, although the term pithomycotoxicosis may be more correct, facial eczema is recognized and widely used in the farming vocabulary and for this reason, this thesis will continue to refer to facial eczema.

Currently, the costs of facial eczema (FE) in dairy cattle are not fully understood. The most recent publication on costs of FE was produced by AbacusBio in 2014 (Dennis and Amer 2014). They estimated that the national economic impact of FE is $30 million annually with zinc prophylactic treatment and if not used the cost increases to $97 million annually.

In comparison to the literature available on production losses in sheep, the figures cited in dairy cows are based on many assumptions from very few published studies (Towers and Smith 1978; Smith and Embling 1983; Towers 1986; Dawson and Laven 2007).

These figures are a gross underestimate given the difficulty in obtaining financial loss figures from subclinical disease in both cattle and sheep. FE is not only important economically but it also significantly reduces animal welfare and is a possible cause of reduced immunological competence in affected animals (Smith and Payne 1991).

It is a commonly held belief that the facial eczema season is getting longer and more severe. Such beliefs have been held since at least the 1970s (Parle and Menna 1978). Potential reasons for this increasing length and severity include global warming, poorer pasture persistence of improved pasture species, over grazing due to increased stocking rates, less hay production spreading seed, cow genetic susceptibility and ineffective zinc supplementation methods.

Whether these beliefs are true or not, they have motivated continued research into a better understanding of the fungus and development of better strategies for disease control.

# Chapter 2

# A review of the disease and its management

## 2.1 THE DISEASE

A description of the effects of FE were published a long time prior to the discovery of the exact cause (Cunningham *et al.* 1942; Clare 1944; Dodd 1959). Mortimer *et al.* (1962) defined FE as an acute obliterative cholangitis to which all other changes are secondary.

After ingestion, sporidesmin appears rapidly in the blood and is concentrated in the bile (Mortimer and Stanbridge 1968). The toxin causes damage to the liver, starting with thickening and oedema of bile ducts and progressing, when severe, to obliteration of a variable number of bile ducts. This obliteration is followed by proliferation of the portal bile ducts proximal to the original lesion, portal fibrosis and replacement of liver cells with fibrous tissue. In addition to the classical obstructive cholangiohepatopathy, intra-lobular biliary hyperplasia and fibrosis, sub-epithelial vascularisation and neutrophilic infiltration of the urinary bladder can be seen (Smith 2000). Thymic atrophy and adrenal hypertrophy can also show in the more severe cases. Grossly, this results in the liver becoming yellow/green in colour and firm in texture with thick and rounded borders (Dodd 1959).

The bile duct occlusion results in substances which are normally excreted in the bile gaining entry to the blood stream. Of particular importance for the clinical signs of FE is the build-up of phylloerythrin, a normal breakdown product of chlorophyll; in sufficient quantities phylloerythrin reacts with sunlight releasing free radicals which cause photosensitivity lesions on the lighter coloured areas of the skin (Clare 1944). The onset of visible symptoms appears to be sudden and often affects a number of animals at the same time. Sheep stamp their feet and shake their heads violently and show obvious discomfort. They will make use of all available shade and often thrust

their heads into hedges. Oedema of the ears, eyelids and lips develops and may extend to coronets and the vulva. Within days oedematous skin becomes necrotic, dries up, and curls away. Icterus is often present and persists for many weeks. In milking cows the first sign of the onset of photosensitivity is often shown with a drop in milk production. The teats and udders become hyperaemic, painful and swollen. Serous discharge will later appear from these regions. Inflammation can extend to the perineal region, muzzle, the area surrounding the eye and the under-surface of the tongue. Dermatitis appears on the white areas of the skin; in severe cases as in sheep, the dermatitis can cause the skin to peel off. Brisket oedema is also a common observation in severely affected cattle as is icterus. Urinary bladder oedema, haemorrhage and even necrosis may occur in such cases (Cunningham *et al.* 1942).

Liver regeneration, which can start as early as 2 weeks after the initial damage from the toxin, may be apparent macroscopically at 6-8 weeks (Manns 1978). This disease has been reported to cause considerable stress as measured by serum cortisol changes (Smith and Payne 1991).

The first bioassay for toxin which was thought to cause FE was the feeding of dried grass samples to guinea pigs (Evans *et al.* 1957). Once a fungus was identified as the cause of facial eczema, it was fed to guinea pigs, who either died shortly after or showed the characteristic liver lesions seen in previous studies in sheep and cattle (Percival 1959). This study then expanded to feeding five lambs with different amounts of fungal cultures for one week. The lambs were slaughtered after 7 days and their livers examined. Of the three lambs fed low levels of fungal culture, one showed no hepatic changes while the other two showed mild degenerative changes of the bile duct. The two lambs fed high levels of fungal culture showed well-defined bile duct damage which was consistent histologically with FE. One lamb was then fed the high dose of fungal culture for 21 days. This lamb developed clinical signs of photosensitivity on day 16 and when slaughtered on day 35 the liver was in advanced stages of fibrosis.

A number of studies since have used purified sporidesmin to elucidate the pathogenesis of facial eczema. Smith (2000) conducted two experiments to determine the clinical and subclinical effects of

different dose rates of oral sporidesmin for time periods ranging from 3-48 days. This study showed that clinical signs of photosensitisation were evident after a minimum of 14 days, consistent with the 10-20 day period reported by (Parle and Menna 1978). Increasing the dose of sporidesmin caused a reduction in live weight, carcass weight and skin weight. There were also pathological changes in the liver, kidney, hepatic lymph nodes, thymus, adrenal glands, heart and spleen. The effects on the internal organs were consistent with those reported in previous studies (Mortimer and Taylor 1962; Smith and Payne 1991), although Smith (2000) was the first to show the increase in weight and oedema of the hepatic lymph nodes . The effects of increasing duration of sporidesmin exposure were similar to those resulting from increased sporidesmin dose.

Although, traditionally, facial eczema has been viewed as a clinical disease with the focus on photosensitivity, the majority of the economic cost of FE results from sub-clinical damage to the liver.

Sub-clinical facial eczema disease in sheep has been shown to decrease fertility (Sheath *et al.* 1987; Towers *et al.* 1987; Moore *et al.* 1990), decrease live weight (Towers and Stratton 1978; Smeaton *et al.* 1985; Smith 2000), and decrease ewe survival (McMillan *et al.* 1988), as well as decrease lambing birth, docking, weaning weights and survival (Smeaton *et al.* 1985; McMillan *et al.* 1988).

The effects of sub-clinical facial eczema have not been studied as thoroughly in dairy cattle. Towers and Smith (1978) studied the effects of purified sporidesmin in three groups of 10 cows. One group received 3 consecutive days of sporidesmin (0.085 mg/kg/day) only, one group received 3 consecutive days of the same dose of sporidesmin as above but 5 consecutive days of zinc sulphate enveloping the sporidesmin dose and the third group was kept as a control. After the initial sporidesmin challenge produced no observable response, other than a transient fall in milk yield, the two groups receiving sporidesmin were dosed again with a dose at 2.5 times the initial rate. After this, transient diarrhoea was observed in about 30-40% of the cows. Milk production in the sporidesmin-only group dropped initially by 90% in comparison to controls and after one month was still only 60% of the controls. The milk production in cattle dosed with zinc and sporidesmin dropped

by 50% in comparison to the controls and recovered back to initial levels within 20 days. In addition, body weight decreased in those dosed with sporidesmin only. All these changes were seen despite none of the animals showing any signs of photosensitisation.

Towers and Smith (1978) suggested that the immediate changes to milk yields could be due to a transient depression of appetite and/or a direct effect of the toxin on the mammary gland as these effects were observed without any detectable liver damage. Facial eczema damage has also been shown to increase the flavour compounds indole and skatole (3-methylindole), which contribute to the 'pastoral' flavour of meat and milk to undesirable levels (Fraser 2006; Fraser *et al.* 2008).

Steffert (1970) postulated that FE damage contributes to culling rates, `unexplained deaths' and metabolic diseases in dairy cows. However, Towers (1978) blood sampled ten full herds to test for serum gamma glutamyl transferase (GGT) activity in May and June of 1976 and the farmers recorded information on stocking rate, production levels, farm area, health events, calving events, oestrus and mating dates from seven of the ten farms. This study showed that 40% of animals sampled showed evidence of liver damage (GGT>30IU/L) with 18% and 4.6% showing moderate (GGT 200-999 IU/L) and severe (GGT >1000IU/L) liver damage. Analysis of the data by what is assumed to be a chi-square analysis did not show a relationship between serum GGT activity and subsequent animal health events or reproductive performance but did show small differences between mean body condition scores measured in May and July, for animals in each health-problem grouping. However, their univariable analysis did not account for many known confounders for health, metabolic and reproductive outcomes such as age, calving date, and condition score at key times of the year so a lack of apparent relationship was only speculative. Furthermore, the relationship between GGT and calving to conception interval did not have the analysis methods provided which may significantly alter the findings in this type of outcome (e.g. mean difference vs survival analysis). Morris *et al.* (2002a) reported the fate of 1500 Jersey sired 1$^{st}$ lactation heifers in 60 spring calving herds after they were blood sampled and tested for GGT in April/May of 1989. Seventy-two percent of the herds contained heifers that had been exposed to FE (GGT>30 IU/L). In the 43 exposed herds, survival of animals to the second lactation was 5.9% and 9.1% lower for animals with a GGT cut-off of >100 IU/L

and >200IU/L respectively. However, for animals surviving at least to the end of the 1st lactation, there was no long term carryover effect of a first-lactation challenge. While the article did not speculate on why the difference occurred, it is likely due to the effect that FE has on the common reasons for death and culling such as health problems, production and reproductive performance.

## 2.2 DIAGNOSIS OF LIVER DAMAGE

As discussed earlier, sporidesmin intoxication causes characteristic liver lesions. A subjective scoring system based on the extent of the damage (McFarlane *et al.* 1959), has been used successfully in experimental studies in both sheep and cattle (Sinclair 1961; Smith *et al.* 1977; Smith and Gravett 1986). This scoring system that grades livers from 1 to 5 based on the presence of lesions (0%,5%, 25%, 50% >75% liver affected) imposed a number of restraints on experimental design , primarily due to the fact that animals had to be euthanized so this limited numbers available to use and the timing of the diagnosis. Furthermore, the subjective nature of the method meant that comparisons between studies and between scorers were of limited value.

Measurement of systemic liver enzyme activity provides a potentially more objective method of detecting liver damage. Both Batsakis *et al.* (1974) and Towers (1975) evaluated, in sheep, the impact of experimental intoxication with sporidesmin on the activity of a range of hepatic enzymes. Of all the enzymes tested, GGT was the only one that met the criteria of elevation being proportional to the degree of liver damage. The base line circulating level in the blood is quite low (0-30 IU/L) normally in very low quantities and persistent as long as tissue damage persists. As a diagnostic test GGT assay also has the advantages that the enzyme is stable during preparation and easily assayed. The finding that GGT elevation was diagnostic of liver damage due to sporidesmin intoxication is consistent with the findings of Ford (1974) that systemic increases in GGT activity were seen in diseases which affected the biliary tract.

Towers and Stratton (1978) showed a high correlation (0.65-0.81) between the increase in GGT activity after experimental sporidesmin poisoning and subjective assessment of the pathological

effects of sporidesmin on the liver of sheep. The greatest discrimination between liver grade groups was obtained when blood samples were collected 2 weeks after the sporidesmin challenge. Samples collected more than 3 weeks after dosing were only suitable to determine between unaffected and sub-clinically affected animals but not accurate enough to distinguish between (or rank) cattle with moderate to severe liver damage as the high GGT activities resulting from severe liver damage had begun to reduce within 21 days of sporidesmin dosing. Blackshaw (1978) showed that in cattle with clinical facial eczema, on average, serum GGT activities were > 45 times the upper limit of the normal range. He also showed that serum GGT activity for approximately up to 33% of animals was raised for four months following clinical facial eczema and mildly raised GGT values were found in apparently healthy cows. Towers and Smith (1978) found a good correlation between liver damage score and serum GGT in 20 mixed age and breed dairy cows ($r^2$ =0.83). Morris *et al.* (2002b) investigated this relationship in cattle that had been genetically selected for facial eczema resistance and concluded that the relationship between GGT and liver injury score had not changed as a result of genetic selection and is still a good indicator of FE damage.

Faull (1986) sampled 15 cows per farm from 88 farms in the Palmerston North region and Taranaki sub region and compared farmer reports of clinical cases with elevation of GGT activity to ≥60 IU/L. At the herd level there was a significant association between the presence of clinical signs and elevated GGT; the risk of finding at least one cow with elevated GGT was 1.8 (95% CI 1.3 – 2.4) times higher. However cows with elevated GGT activity were still reported on 24/50 farms with no reports of clinical cases, and on the 38 farms which reported clinical cases, testing of GGT activity found no evidence of subclinical disease on 6 farms

Overall, Faull (1986) reported that there were >13 cows with elevated serum GGT activity (subclinical FE) for every clinical case of FE reported. However, at the farm level there was a significant variation in the ratio between reported clinical cases and the proportion of animals sub-clinically affected. For example, Faull (1986) reported that for farms with no clinical cases reported by the farmer, the proportion of sub clinically affected cows ranged from 0 to 20%, while on farms with 5% of clinical cases the proportion was 15 to 55%.

Despite the correlations, it is still important to consider that not all GGT values are the result of liver damage from facial eczema. Ingestion of brassicas around the same time as the facial eczema season can mimic both the photosensitivity lesions and GGT results (Collett *et al.* 2014). Histopathology of the liver when uncertain is still considered a definitive diagnosis to determine between the two as the histopathological changes seen in the liver of subacute cases of the respective diseases are unique and diagnostic (Collett 2014). Other possible causes of raised GGT are muscle damage, spring eczema, ragwort toxicity, fasciolosis, lipidosis, kidney damage and chronic ill thrift (Blackshaw 1978).

## 2.3 THE FUNGUS AND IT'S TOXIN

A microbiological origin of the toxin which caused FE was first suggested at least as early as 1942 (Cunningham et al 1942) but chemical extraction and identification of the toxin itself was not achieved until the late 1950s (White 1958) with subsequent work showing that it was a toxin produced by a fungus (Percival 1959; White 1959). This fungus was originally identified as *Sporidesmium bakeri* but following further investigation it was classified as *P. chartarum* (Ellis 1960).

*P. chartarum* is a dothideomycete in the phylum Ascomycota. The exact taxonomy of *P. charatarum* is currently uncertain. A potential sexual form of *P. chartarum* was identified in South Africa by Roux (1986). This teleomorph was classified as *Leptosphaerulina chartarum*; it is apparently rare and has not been reported in New Zealand (Di Menna *et al.* 2009). However, phylogenetic analysis suggests that *P. chartarum* does not cluster closely with the type species of its genus (*P. flavus*) or with *Leptosphaerulina spp.* (Phookamsak et al. 2013). Much further work is required to effectively establish the taxonomic relationships of *P. chartarum*.

Dingley (1962) described the key morphological features of *P. chartarum*. It has short conidiophores which each bear one conidium (asexual non-motile spore). The mature conidia are dark brown or black, dry and dispersed in air (Dingley 1962). It is the conidia which contain the sporidesmin and the fungus does not produce sporidesmin until it begins sporulating (Clare and Gumbley 1962; Davison and Marbrook 1965).

A comprehensive study of the ecology of *P. chartarum* by Brook (1963, 1964) investigated the conditions which were optimal for the growth and sporulation of *P. chartarum*, at first within a controlled laboratory environment and then in the field. This work showed that *P. chartarum* is an extremely variable species which is influenced greatly by conditions such as temperature, moisture and substrate on which is grown (Dingley 1962; Brook 1963).

***Temperature***

Spores of *P. chartarum* germinate freely from 12 to 27 °C but germination is inhibited at 3 °C. The temperature at which the highest proportion of spores germinated was 24 °C. For maximum infection of leaf material, Brook (1963) showed that this temperature needed to be combined with 100% relative humidity. At 24 °C and 100% humidity colonies of *P. chartarum* produced fully formed conidia within 48 hours of inoculating the grass. However, in the field, it usually takes 3-5 days for the whole cycle from infection to sporulation to be completed. Sporulation was also found to be optimal at 24°C. Although continuous optimum temperature favoured maximum sporulation, it was still possible to have substantial spore production when the optimum temperatures were present for only 6-8 hours a day.

***Moisture***

Moisture requirements for *P. chartarum* were at or near 100% relative humidity. In the field this can be achieved following rain or dew.

Much of the information on optimal temperature and humidity provided by Brook 1963 were based on data obtained in the laboratory but observations in the field (Brook and Mutch 1964) showed that the most suitable weather conditions for *P. chartarum* were full, overcast, showery conditions lasting for at least 3 days with mean temperatures of 18°C.

***Substrate***

*P. chartarum* is only capable of saprophytic growth, but has been recorded growing on the debris of a large number of plant species (Dingley 1962). In the laboratory, *P. chartarum* has been shown to grow and sporulate many different grass and legume species (Dingley 1962). In the field competition from other saprophytes and the quantity of plant debris are significant influences on the number of spores found in a pasture. In the field, the amount of debris presented by a pasture species influences the development of *P. chartarum* more than just the inherent quality of the debris; e.g. clover species are very successful at growing the fungus in the laboratory but in the field they do not have a lot of dead material for the fungus to grow on, while tall fescue has a higher branching structure, more resistance to microbial degradation of old stems, a lower tillering rate and more longevity of leaves and tillers than perennial ryegrass (Hume and Brock 1997), thus reducing the quantity of available dead matter at the base of the sward for the fungus to live on. Attack of pasture plants by pathogens (black beetle, leaf rust) can produce more debris for the fungus as does differences in palatability of areas of the pasture. Under rotational grazing, livestock remove a greater proportion of the material present at urine-patches than at inter-excreta sites (Keogh 1975, 1986) and consequently the close grazing which occurs as the urine-patch sites can contribute disproportionaely to the ingestions of *P. Chartarum* spores. The presence of white clover also influences feeding behaviour. The ryegrass present at sites containing white clover is grazed much closer than is ryegrass at either urine or inter-excreta sites (Keogh 1975). This can influence spore counts by reducing the amount of dead matter through close grazing (Keogh 1975). Topping of pasture to increase quality increased the activity of *P. chartarum* in trial plots and Thornton and Sinclair (1960) and Sinclair (1961) demonstrated the severity of facial eczema increased by topping and leaving dead pasture.

The spores of *P. chartarum* are not only found on dead material as dispersal of spores in the air above the pasture can move spores to green leaves Although there is some adherence between spores and substrate material due to spicules on the surface of the spore (Bertaud *et al.* 1963), Smith and Crawley (1964) showed that large water (or rain) drops could be a potent means of detaching spores from the plant material (litter) on which they were produced. Water films on

herbage leaves can also aid in moving spores up or down leaves for short distances. Smith and Crawley (1964) showed that the air-shock waves and turbulence following water drop impact would also be adequate for local spread of the spores to surrounding green leaves even the absence of surface wind currents. This finding is supported by Hirst and Stedman (1963) who showed there were transient increases in the concentration of some dry airborne spores coincident with the start of rain suggested that the first raindrops to wet surfaces might disperse spores other than in splash droplets or by wetting fructifications (Hirst and Stedman 1963).

***Toxin***

The toxin of *P. chartarum* was isolated, characterized and was named "sporidesmin" in 1959 (Synge and White 1960).

Sporidesmin contains a disulphide group and it is the reduced (dithiol) form of sporidesmin which has been shown to readily undergo auto-oxidation in vitro, thereby generating a superoxide radical ($O_2^-$). This is a cyclical process, such that a single molecule of sporidesmin can generate many molecules of superoxide. The superoxide radical then undergoes dismutation to hydrogen peroxide:

$$2O_2^- + 2H^+ \longrightarrow H_2O_2 + O_2$$

In the presence of certain metals such copper, this reaction is catalysed and hydrogen peroxide reacts with superoxide to yield the hydroxyl radical:

$$O_2^- + H_2O_2 \longrightarrow O_2 + 2OH^-$$

The superoxide, hydrogen peroxide and hydroxyl radical are known collectively as 'active oxygen species' (Munday 1982). Superoxide radicals are the cause of the pericholangitis seen in cases of FE (Munday 1982). The experimental pathology of poisoning with sporidesmin has been studied in animals and in vitro (Mortimer 1963; Mortimer and Collins 1968; Mortimer and Stanbridge 1968) and all showed that sporidesmin is not specifically a liver toxin. Mortimer and Stanbridge (unpublished) claimed that at low concentrations it is a highly potent inflammatory substance, similar to histamine but at higher concentrations it causes irreversible changes in cells which results

in their destruction. Mortimer and Stanbridge (1969) showed that the sporidesmin requires no metabolism by the liver to exert its toxic effects. The toxin is broken down in both UV light and water from rain or dew (Di Menna *et al.* 1970). In rainy weather it is likely that only newly produced conidia will contain significant amounts of sporidesmin (Marbrook and Matthews 1962).

Different strains of *P.* chartarum vary greatly in their ability to produce sporidesmin and spores and despite many differences in the methods people have used in the media, incubation times, temperatures and method of sporidesmin assay, the overall findings are that there is a relationship between production of sporidesmin and rate of sporulation (Dingley *et al.* 1962; Davison and Marbrook 1965; Di Menna *et al.* 1970). Other factors that can influence sporidesmin production include ultraviolet light which causes stimulation of sporidesmin production in the growing fungus but destruction in aqueous solutions (Di Menna *et al.* 1970); the substrate on which certain strains are grown on in the laboratory (e.g. Halder *et al.* (1981) reported that rye corn produced high concentrations of sporidesmin (Halder *et al.* 1981); and temperature (temperatures >24°C reduced sporidesmin; (Davison and Marbrook 1965). The effect of time after sporulation on concentrations of sporidesmin is not known.

New Zealand has a uniquely high risk of sporidesmin intoxication because the majority of isolates of *P. chartarum* produce sporidesmin. Collin *et al.* (1998) found that 336/391 (86%) *P. chartarum* isolates from New Zealand produced sporidesmin, in comparison to 138/207 67% from Australia and 1/51 2% in Brazil. The presence of non-sporidesmin-producing (non-toxic) strains of *P. chartarum* gave rise to the idea of using non-toxic strains to compete with the toxin-producing strains. Collin and Towers (1995b) found that when a sporidesmin-producing strain of *P. chartarum* was grown in co-culture with a non-toxic South African strain the total spore production was below that expected from the proportion of each strain present. In two out of their four experiments, the non-toxic strain completely over grew the toxic strain. Collin *et al.* (1996) showed that lambs dosed with the non-toxic isolate, at thirty times the rate required to produce FE with the sporidesmin-producing isolate, showed no observable toxic effects. Fitzgerald *et al.* (1998) applied non-sporidesmin- producing New Zealand isolate to 24 5x5m plots of ryegrass-white clover pasture. Four control plots were left

untreated. Spore counts were determined by the wash method, sporidesmin concentration was determined by ELISA (Collin *et al.* 1995) and collection of individual fungi was undertaken for 19 weeks after application. For the first three weeks mean spore counts were higher on the treated plots to the controls but the sporidesmin levels were always higher on the control plots. The percentages of non-sporidesmin-producing isolates recovered from the treated plots declined from 90% to 54% after 19 weeks of the trial and after 15 months, only 4% of the isolates left were non toxigenic.

The apparent protection against sporidesmin in mice vaccinated with 2- amino-5-chloro-3,4-dimethoxy benzyl alcohol (ACDMBA) bacteria complexes (Jonas and Erasmuson 1979) gave hope to the idea that sheep may be able to be vaccinated to protect against sporidesmin. However Fairclough *et al.* (1984) found that in contrast to the mouse immunisation experiments, despite the fact that immunised ewes had a measurable quantity of circulating anti-dinitrophenol antibodies, they showed a more severe reaction to the toxin (as assessed by deaths, liver and urinary bladder injury scores, and bilirubin and cholesterol measurements) than did the non-immunised control ewes.

## 2.4 CONTROL AND PREVENTION STRATEGIES

There are a number of strategies available for control and prevention of facial eczema. They are: a) recognise and avoid the toxin; b) supress toxin production; c) protect the animals if toxin is ingested; and d) breed for FE tolerance.

***Recognition of toxic pastures***

Two different methods have been used to help farmers identify toxic pastures. The first method is based on the identification of weather conditions which are suitable for growth and sporulation of *P. chartarum* and the second is measurement of spores either in the pasture or in the faeces of animals grazing that pasture.

The variability in conditions giving rise to facial eczema outbreaks was recognised as early as 1940 (Cooper and Walker 1940). The first fifty years of facial eczema research helped to define the short term weather conditions which were associated with an increased risk of FE. Spore numbers on pasture are the sum of their production by the fungus and destruction or removal (Di Menna and Bailey 1973). Both of these processes are weather dependent. Spore production is influenced by temperature and humidity (particularly rainfall and dew production) (Brook 1963), as is the loss of spores through germination (Mitchell et al 1961). The other key factors which influence spore loss such as desiccation by wind and translocation by rain, as well as ingestion by macro and micro-fauna are also weather dependent (Di Menna and Bailey 1973).

Crawley and Woolford (1965) produced a FE predictor based on weather records, growth patterns of *P. chartarum,* grass minimum temperatures and rainfall over 3-day periods. It incorporated the findings from Mitchell *et al.* (1959) who showed that spore counts did not rise rapidly in the first warm, moist periods of the season as *P. chartarum* populations needed time to establish and because of early competition in the pasture litter with earlier-established saprophytic moulds (Brook 1963). The aim of the predictor was to give farmers a method of determining pasture toxicity on their own properties by using grass minimum temperatures and rainfall. However later trials showed that the association between the predictor and spore counts was not sufficient for the predictor to be used as an early warning system for FE (Parle 1967; Di Menna and Bailey 1973). The source of this variability is probably the limited data that were included in the predictor; for example, measurement of grass minimum temperature gives some indication of heat at the base of the pasture but length of time at 100% humidity is affected by temperature, rainfall, day length, cloud cover, soil moisture and wind, all of which are not measured by the farmer. Di Menna and Bailey (1973) tried to expand on the weather predictor by examining additional factors that might influence spore numbers in pasture. They found that spore counts at sites facing to the north and sheltered by a hedge from the south were almost always higher than those on neighbouring flat, unsheltered sites. They also showed that shelter increased spore counts, but aspect alone did not influence spore count in a consistent pattern across farms.

Although these findings are consistent with our understanding of the biology of *P. chartarum*, these conclusions were from limited data. The combined effect of aspect and shelter was examined using repeated spore counts from two sites on one paddock on one farm; the effects of shelter were compared on single paddocks on three different farms and the effect of aspect alone on 12 paddocks from two farms (10 on one and two on another). Furthermore there was no reported statistical analysis of the data. Nevertheless the data show that warning systems based on location and weather were only moderately predictive and individual farm spore counts were necessary for avoidance of toxic pasture.

Counting spores is not a direct measure of pasture toxicity, but in vitro studies have shown that the amount of toxin produced is correlated to the number of spores in the pasture Collin *et al.* (1995). However, this association is not completely consistent; Clare and Gumbley (1962) showed that there could be a 3 to 5-fold variation in sporidesmin content in spores from one area during the FE season and exposure of spores to UV light and water can also alter the sporidesmin content of spores (Clare and Gumbley 1962; Marbrook and Matthews 1962). Another source of variation between spore counts and pasture toxicity is the method used to count the spores in the first place. All the experiments that compared sporidesmin concentration with spore counts used a version of the spore counting method designed by Thornton and Sinclair (1960) but all had variations in the quantities of grass, grass lengths, aliquot quantities and shaking/squeezing method along with time for shaking/squeezing used.

Nevertheless as direct measurement of sporidesmin concentrations is currently not feasible in a timely manner, particularly in a general veterinary practice setting; spore counting remains the best option for identifying the risk of FE in grazing animals.

Dry (trap) or wash counting methods have been used over the years to count spores of *P. chartarum.* The volumetric spore trap method was first reported by (Brook 1959). This involved a machine with rotating blades being pushed over a strip of pasture. As it went along the blades threw up spores from the pasture; these were sucked into an impactor and then on to petroleum jelly coated

microscope slides. Modifications were made to the machine;(Faull 1986) reported using a trap with a container on skids, that was fitted with a blower that disturbed spores on the grass and caused them to deposit on the microscope slides. In addition to the requirement for a bespoke machine, the key disadvantages of this method were that spore counting during wet weather or in the morning when dew was present was not possible. Dry spore counting has thus been replaced as a predictor of FE risk by wash counting.

Thornton and Sinclair (1960) were the first known researchers to develop a "wash technique" for assessing spore numbers. Twenty-five grams of fresh grass, cut at a height of 2.5 cm with hand box shears, was added to tap water (250 ml plus 1 drop of a sodium dodecyl benzenesulfonate-based detergent ['Teepol']) and shaken vigorously by hand for 1 minute in a jar. The contents were then strained through a 2 mm sieve. Counts of spores were made on the washings with the use of a Neubauer counting chamber. In each chamber the spores in five 1 $mm^2$ grid squares (centre and four corners) were counted.

The statistical analysis of this technique was limited; the authors reported that its accuracy and reproducibility were good, but no details of the analysis used or the outcomes of that analysis were reported. However, they did report that there was a reasonable correlation (r =0.645) between their spore counts and the then current test for sporidesmin (the 'beaker' test).

This initial technique has been modified over time. Di Menna and Bailey (1973) used 25 g samples of well-mixed pasture leaves, and mixed them, using a mechanical shaker, with 250 mL of water for 5 minutes. The wash water was then placed, without preliminary straining, onto haemocytometer slides and the spores in 2 $mm^3$ counted. Each spore seen was stated as representing 5 000 spores/g of leaf (wet weight). They reported that the mean difference between counts of the same wash water (n=7 samples) was 9 000 spores/g (range 0—15 000). They also compared the results from 12 grass samples where two separate preparations were made from each samples. In this case the mean difference was 8 000 spores/g (range 0—25 000). However, during years 1971-72 of this trial, old, partially disrupted spores were not counted due to claims that they were 'probably non-toxic'. It

is unclear whether they disregarded 'non-toxic' spores during the reliability testing of this trial; however, there is no discussion in this paper or any others about systematic identification of such spores and therefore such assumptions are likely to be subjective and not reliable.

Collin *et al.* (1995) used samples of pasture collected from 5 sites in a paddock cut at 1cm above the ground. Samples were mixed and cut to 4 cm length then 15 g was placed in a plastic bag with 150 mL of tap water and squeezed every few seconds for a minute. An aliquot of this water was used to put under a microscope in counting grids. They compared the relationship between spore counts and sporidesmin concentrations (determined using an enzyme immunoassay) in pasture using samples taken from eight paddocks, twice a week for a period of 4 months from February to May 1994. They reported that there was a significant linear relationship between sporidesmin concentration and spore count ($r = 0.66$).

The spore counting technique recommended by Oldman and Di Menna (1983) is the method which is currently used by the majority of veterinarians, farmers, laboratories and researchers. In this method, 200 g of pasture is collected by walking diagonally across a paddock and stopping 10 times to collect approximately 20 g cut at ground level. These samples are then gently mixed inside the collection bag, and 60 g of pasture then removed. This is added to 600 mL of water in a plastic container. The container is then shaken vigorously for 3 minutes, the pasture removed, and an eye dropper used to collect a sample of the solution while gently agitating the container back and forth. A cover slip is then applied over the grids of the haematocytometer and both sides of the slide are filled with the sample solution. Spores are counted using a microscope at 100x magnification. Depending on the depth of the grids the total pasture spore counts/g pasture are estimated by multiplying the number of observed spores by 5 000 or 10 000. The method does not adjust for major differences in dry weight which may alter the overall spore count estimates, however if samples are collected at the same time in the morning when dew is still present on the pasture then crudely a similar weight may be achieved.

While pasture spore counts give an indication of the rises and falls in the number of spores on a pasture, the relative risk of FE is also influenced by the variability of spore counts across a paddock, grazing preferences (urine patches vs dung patches; (Keogh 1986), stock type, age and grazing pressure. Pasture spore counts may therefore not accurately represent sporidesmin intakes; this led to the suggestion that measuring rumen or faecal spore counts could be a better measure for estimating FE risk. Smith *et al.* (1987) evaluated rumen and faecal spore counts in 3 groups of 18 sheep under different grazing pressures. This work showed that although both faecal and rumen spore counts did reflect changes in pasture spore count, within each grazing group faecal spore counts gave a lower coefficient of variation than rumen counts and the coefficients of variation for both rumen and faecal spore counts were lower at higher spore counts. The best correlations of faecal spore counts with pasture spore counts were when they were sampled on the same day and at a higher grazing pressure (probably due to faster transit through the gut). In a subsequent study Keogh (2001) showed that there was a good positive relationship between faecal spore load and a GGT index ($r^2 = 0.86$).

Nevertheless, while faecal spore counting may be useful in estimating the sporidesmin exposure of particular animals, the value of using this method for predicting danger periods has not been demonstrated. In particular, there is still insufficient information to guide the interpretation of faecal spore counts and no data on the reliability of faecal spore counting.

Since the early 1960's, district warnings of facial eczema danger have been issued by local warning committees (Parle and Menna 1978). The disadvantages of these warnings is that spore counts can change both with time and distance (Di Menna and Bailey 1973; Parle and Menna 1978). A survey conducted in June and July of 1986 on 122 farmers in the Palmerston North and Taranaki regions (Faull 1986) was undertaken to assess the effectiveness of facial eczema warnings. Most farmers had access to facial eczema warnings (97.5%) which at this time were mainly by radio or newspaper. Most farmers felt these warnings were helpful but few farmers took action before the first warning was issued, indicating little use of preventative measures which need to begin some weeks before a

danger period. About 75% of all farmers in the area took some action as a result of the warnings but very few monitored the risk further.

The conclusion from studies of spore counting and FE particularly that of Di Menna and Bailey (1973), is that while district spore counts have some benefit in reducing the incidence of disease, variability between paddocks and farms means that spore counts on individual farms are needed. Furthermore, even though spore counting has been widely used as a predictor of FE, there are still major areas where more data is needed. Firstly, there has been very little work to quantify the sources of variation within and between paddocks. Secondly, there has been an almost complete absence of data on the reliability of spore counting i.e. How accurately an individual spore count from a single aliquot of wash water estimates actual pasture spore count.

***Avoiding the toxin – grazing management and pasture species***

Because of the saprophytic nature of *P. chartarum*, the relative number of spores on green leaves is lower in tall pasture than short pasture (Smith *et al.* 1962; Brook 1963). More cases of facial eczema were seen when sheep were grazed on short rather than long pasture (Smith *et al.* 1963).It is thus logical that spore intake could be reduced by grazing management strategies such as lower stocking rate, supplementing with other feed or trying to improve pasture quality.

A study by (Brook and Mutch 1964) attempted to assess the impact of grazing management on FE in grazing sheep. Four different management options were investigated: normal stocking (5 hoggets/acre set stocked), heavy stocking (10 hoggets/acre set stocked), heavy rotation stocking (10 hoggets/acre) and heavy set stocking on irrigated pasture (12 hoggets/acre). Results showed that heavy stocking to control dead matter was effective if the pasture was green and growing through regular rain, or in this case, irrigation. Heavy stocking on its own reduced the amount of dead matter and the severity of the facial eczema but did not prevent it entirely and ultimately the rationed feed affected the growth rates of the hoggets. Low stocking rates resulted in the most facial eczema damage due to large accumulation of dead matter. However, these results need to be interpreted with care as the conditions over the trial period were similar to a drought and some trial paddocks

had a lot of insect damage to the roots of the grass. Therefore, all pasture that was not irrigated was brown and dying, regardless of stocking rate. Furthermore, objective measurements of pasture such as height and composition were not used when comparing FE outcomes.

The practical challenge with intensive grazing of pasture is that the animals are forced to eat closer to the forage base, which is where the majority of the fungus and concentration of spores is found. (Campbell 1970). If instead of intensive grazing, farmers top pasture, it helps remove the dead and unpalatable pasture but provides a nice bed of dead material for the fungus to grow on when weather conditions permit (Parle and Menna 1978). Regardless of intensive grazing or topping, there is always enough dead material, often attached to leaf sheaths to support fungus growth to dangerous levels (Parle and Menna 1978). This suggests that even though grazing pressure and topping may help reduce the dead matter, it is only a tool that should be seen to help with FE management rather than be used as a sole means of management.

The role of pasture species in the development and control of facial eczema was initially researched on multiple paddocks in Northland, Waikato and Palmerston North from 1997-2000. Chicory (*Cichorium intybus*), clovers *(Trifolium pratense and Trifolium repens)* and tall fescue *(Festuca arundinacea)*-based pastures were first compared to ryegrass dominant pastures *(Lolium perenne)* by assessing faecal spore counts alongside serum GGT activity and liver damage recorded at slaughter in animals that were grazing paddocks made up of different pasture mixes.

Pastures were also compared by inserting leaves of ryegrass, cocksfoot *(Dactylis glomerata),* browntop *(Agrostis tenuis),* Yorkshire fog *(Holcus lanatus),* chicory, red clover, white clover, lotus *(Lotus pedunculatus)* and tall fescue into the base of plots growing these species in March 1997. Leaves were sequentially harvested on six occasions during the following 8 week period and *P.chartarum* spore loads were determined (Keogh 2001).

Results from these studies suggested chicory, red and white clover, lotus and tall fescue supported low levels of *P. chartarum* while ryegrass, cocksfoot, browntop, and Yorkshire fog supported high levels of *P. chartarum*. From the grazing trials, higher intakes of *P. chartarum* spores were recorded

on browntop, cocksfoot and ryegrass-based pastures. Spore intakes were consistently low on chicory, red clover and tall fescue pastures.

However, the faecal spore count data that the conclusions were made from were taken from a number of different paddocks in three different geographical locations in New Zealand over three different years without any statistical analysis. Spore load results from inserted leaves had few measurements to compare as after harvest 3 out of 8 of the inserted leaves had decomposed or been removed by earthworms.

***Supress toxin formation***

Fungicides act by inhibiting germination of spores present at the time of spraying. Preliminary experiments completed by Janes (1962) found no consistent evidence that three types of fungicides controlled *P. chartarum* on ryegrass litter. In the late 1960s thiabendazole and later other substituted benzimidazoles were found to reduce spore numbers for up to 6 weeks after application (Parle and di Menna 1972b, 1972a). Spores were counted using the Brook volumetric spore trap technique (Brook 1959). This study lacked data on spore counts prior to the application of fungicide (spore counts were measured three times a week but only after fungicide application), so the conclusion that fungicides were effective was based on relative reduction in spore counts on treated pasture compared to control. Such figures could be strongly influenced by underlying differences between pastures in compatibility with the requirements for growth of *P. chartarum.* Furthermore the 6 week figure had limited external validity as it was strongly influenced by the effect of time on spore count in those paddocks in that season.

The two best active ingredients trialled (thiabendazole and benomyl) gave relative spore counts of 40-50% and 25-40% of the control spore counts. Simulated rainfall of 25-50mm within 3 days of application removed all effects of the treatment. No evidence was found that extended exposure to sub-lethal concentrations of thiabendazole conferred any detectable resistance on *P. chartarum* . Furthermore, under field conditions resistance is less likely to occur than in laboratory tests because

the fungicide is applied only to small areas during a few months of the year, so there would be little selective advantage for resistant strains of the fungus.

These considerations led to the trials throughout the North Island in 1968 that looked at different application rates of two different types of fungicide in comparison to two control plots at 6 different locations in New Zealand. Spore counts were measured prior to application and during the trial using the volumetric spore count technique (Brook 1959). Overall it was noted (numbers were not presented) that spore counts in the treated plots were very low in comparison to the control plots which ranged from 30,000-70,000 spores/cubic metre. The incidence of liver damage in the lambs grazing control plots across all studies was higher than in treated plots. A similar study completed in 1971 compared different rates of application of three different types of fungicides including thiabendazole (Campbell *et al.* 1971). There were two unsprayed control plots included in each trial and effectiveness was measured by assessing liver damage in animals that grazed those plots for a period of 6 weeks. Campbell *et al.* (1971) found that fungicides were only moderately effective at reducing the incidence of liver damage and there was no difference in liver damage between different application rates. However, these results could have occurred because fungicides were applied to high spore count pasture where the reduction in spore counts was not sufficient to decrease spore numbers to non-toxic levels prior to grazing or the timing of rain after application.

The application of lime fertilisers on pastures as a protective measure against facial eczema has been debated for more than 75 years (Anonymous, 1970). Lime fertilizers are routinely applied to agricultural soils in New Zealand to raise pH into the range of 5.7-6.5 which is considered optimal for pasture growth (Haynes and Naidu 1998). The anecdotal belief from many farmers is that the application of lime on pastures helps prevent the growth of *P. chartarum* fungus, and/or that it helps decrease pasture spore counts, if they are present. The theory proposed is that the change in pH of the soil may alter the growth and sporulation of the *P. chartarum* fungus as soil pH can influence biomass composition of fungi and bacteria (Bardgett et al. 2001; Fierer and Jackson 2006; Rousk et al. 2009).

A widely disseminated research paper (Grierson 2007) concluded that the application of 2.5t/ha of lime caused a reduction in pasture spore counts 7-14 days after application. The lime was applied to one 20 x 5m plot on a Katikati deer farm and the spore counts compared to those from an adjoining untreated control plot. Pasture sampling was then carried out weekly/fortnightly for two months starting 7 days after application of lime. This was carried out for 3 years, starting in 2005. In each year, lime was applied on a different plot at approximately similar dates to previous years. In addition, in 2007, spore counts were collected from the previously treated plots and compared to the control plot for that year to identify if there were residual effects of the application of lime. Grierson concluded that lime application reduced spore counts within 7 days of application and that this effect lasted for up to 2 years.

However, as the initial pasture spore counts for the treatment and control plots were not recorded before the application of lime, it is quite possible that, as there was only one control and one treated plot each year, the differences between treated and control plots were due to pre-existing differences in spore counts rather than treatment, especially since the same control plot was used over the 3 years of the study. To the best of our knowledge there have been no other controlled studies investigating the effects of lime on pasture spore counts.

***Protecting the animal***

It was noted in Mortimer and Collins (1968) that even small alterations to the molecular structure of sporidesmin lessens or destroys its toxicity. Chvapil (1973) suggested that zinc is important in the stability of macromolecules. Zinc forms a stable mercaptide with reduced sporidesmin, removing it from the auto-oxidation cycle that leads to the cascading generation of reactive oxygen radicals. (Munday 1982; Munday 1984; Henderson *et al.* 1995).

The use of high intakes of zinc was first discovered in the early 1970's by Gladys Reid of Te Aroha who received a large amount of criticism from academics for this theory. However it was later shown in Smith *et al.* (1977) that zinc sulphate, administered in a 5-day period bracketing a 3-day period of sporidesmin dose to sheep, gave protection from the effects of sporidesmin when compared to

control groups who were given sporidesmin without zinc. Protection increased but at a diminishing rate with increasing dose rate of zinc. This study also showed that urinary bladder and liver lesions were similarly prevented indicating that the zinc protective effect was not liver specific.

A similar effect was shown in cattle when Towers and Smith (1978) drenched a zinc sulphate solution concurrently with sporidesmin and Smith *et al.* (1978) administered zinc sulphate during a phase of mild natural facial eczema outbreak. Both gave significant protection against the toxin. Towers and Smith (1978) however showed that concurrent zinc sulphate administration gave apparently complete protection against a low sporidesmin dose but only partial protection with a sporidesmin dose 2.5 times higher. The zinc dose used in this experiement was over 20 times that of the normal zinc intake requirement for grazing ruminants (Towers 1977a). Studies done in rats (Towers 1977b) and sheep (Smith *et al.* 1977), indicate that such high doses are necessary to achieve significant protection.

The protective effect of zinc was later confirmed to be related to its ability to inhibit the generation of a superoxide radical by sporidesmin (Munday 1984). It also has the ability to inhibit intestinal absorption of copper which catalyses the reaction (Munday 1985).

Various routes of zinc administration are now used by farmers. The most commonly used methods are zinc sulphate in drinking water and zinc oxide in drench, added to feed or formulated into a slow release capsule. It been recognised that control of facial eczema is achieved by giving zinc daily and before pasture becomes toxic (Smith and Embling 1999).

The effectiveness of administering zinc (Zn) using a continuous supply of zinc sulphate through the water troughs versus daily oral drenching with zinc oxide was compared in Morris (2013). Responses in daily milk yields and concentrations of zinc in the blood were recorded twice weekly over four weeks. The trial showed that serum zinc was elevated consistently by both treatments but drenching gave consistently higher serum zinc concentrations after 3 days of dosing in comparison to 10 days of trough treatment and that there was less variability between cows in serum zinc concentration when they were drenched rather than supplemented via the water.

Zinc-containing intra-ruminal devices have also been developed for sheep and cattle over recent years. They consist of either a zinc oxide core with a water impermeable coating or a bolus made from elemental zinc metal powder. They have been shown to be highly effective in raising both serum and faecal zinc concentrations in sheep (Munday *et al.* 1997; Bennison *et al.* 2010) and cattle (Munday *et al.* 2001). However Munday *et al.* (2001) showed that while the concentration of zinc in the faeces was similar between the zinc oxide and elemental zinc bolus, the zinc concentration in the serum was considerably higher for the zinc oxide bolus between 7 to 56 days after administration. Smith *et al.* (2010) showed that in adult cattle the serum zinc concentration had returned to near baseline by 28 days giving a much shorter duration of treatment than that shown in sheep or the work previously done with calves (Munday *et al.* 2001).

Although in-feed supplementation has been commonly used as part of FE prevention, there have not been any published studies on the efficacy of zinc in feed as a means of FE control.

Providing high concentrations of zinc to cattle does not necessarily mean that it will protect against facial eczema. When zinc is provided in the water, consumption of water decreases markedly. Smith (1980) showed this when providing groups of yearling cattle 3 different zinc concentrations in the water in comparison to a control. Over the high, medium and low zinc concentrations he found that water consumption was 54, 35 and 8% less than the controls. The effect was greatest early in the trial. Over a total of 61 treatments (30-31 days would have been the expected number of days) there was no effect of zinc at all (as determined by weekly serum blood zinc concentrations). A new break of grass or rainfall further decreased consumption of water from all groups, with the effect being most noticeable and long lasting in the group that had the highest zinc concentration. High zinc concentrations were shown to affect weight gain and cause a small degree of pancreatic damage. However, it is not just the presence of zinc that can cause differences in water consumption. Wright *et al.* (1978) showed that while the addition of zinc sulphate did not affect water intake of the 48 trial animals in their study, the water intake varied up to 4-fold between animals and was highly affected by rainfall. Volume of water drunk has also been shown to be significantly and positively related to the dry matter(DM) percentage of the herbage, protein level in the feed, the air

temperature and the daily hours of sunshine and negatively related to daily rainfall (Phillips 1968; Castle and Watson 1973). As *P. chartarum* spores numbers rise rapidly after rain, the need for Zn consumption is greatest at the time when Zn consumption through the water is at its lowest.

If copper supplementation is provided at the same time as zinc supplementation, this may reduce the efficacy of zinc in protecting FE (Dawson and Laven 2007).

***Zinc toxicity***

High dose rates of zinc are required for protection against facial eczema but one of the dangers of this method of control is the relatively low margin of safety (Smith 1977, 1980; Smith *et al.* 1984). If a controlled release device is broken on administration, zinc toxicity will most certainly occur. The toxic effects of zinc salts are well known and include malfunction of digestive organs such as the rumen and pancreas (Ott *et al.* 1966a; Ott *et al.* 1966b; Ott *et al.* 1966c) with ruminants, apparently, being the most susceptible species. Smith (1977) showed that animals receiving the highest daily dose of zinc (180 mg Zn/day) suffered severe diarrhoea with a rapid decline in body weight preceding death at days 7 and 8. Animals receiving lower dose rates (60mg Zn/day) all had similar clinical signs but over an extended period. Pathological findings were necrosis of the mucosa in the oesophagus, rumen, abomasum and all had pancreatic lesions and loss of pancreatic weight. Pancreatic damage from zinc toxicity is greater in sheep receiving zinc after previous sporidesmin-induced injury (Smith and Embling 1999).

Zinc can also interfere with the metabolism of other elements. Increased zinc intake can decrease the absorption and hepatic storage of copper in sheep (Bremner *et al.* 1976) and lambs (Rounce J.R 1998). Similar effects have been shown in cattle. Towers *et al.* (1981) showed that zinc sulphate added to drinking water for cattle to provide 12-15mg Zn/kg live weight for 84 days caused a marked decrease in copper (Cu) in the plasma. Smith *et al.* (2010) found that high zinc levels used in the prevention of FE in dairy cows had little effect on concentrations of Cu in the liver when the Cu intake was low, but decreased the efficacy of a Cu supplement by approximately 50%. Clinical hypocalcaemia in cattle can also be caused by high doses of zinc (Smith *et al.* 1984).

The use of zinc salts at high levels can result in unacceptably high tissue levels of zinc. Residues of zinc in animal products may exceed the recommended dietary intake of zinc (Anon 2003) and may have environmental concerns with leaching into water ways.

***Breeding***

Liver injury score in sheep was found to be a heritable trait (0.42 ± 0.09), based on 160 sire-progeny groups scored (Campbell A.G 1981). After this GGT was used as a measure of liver damage and when sheep were dosed with sporidesmin under the same conditions there was considerable variation in the response to the dosing (Smith and Gravett 1986; Smith 2000). Similar effects have been observed in cattle (Towers and Smith 1978). Smith and Gravett (1986) observed the differences in GGT and liver score after artificially dosing 16 pairs of twin cattle. This showed that the variance in response to sporidesmin challenge is much less within twin sets than between sets, suggesting that more of the overall variance is due to the genetic rather than the environmental component.

The development of experimental FE resistance and FE susceptible lines of Romney sheep began at Ruakura in 1974. Since then genetic responses have been described in detail and have shown a heritability of estimate for $\log_e$ GGT of 0.45 ± 0.03 (Morris *et al.* 1989; Morris *et al.* 1995). A performance test is available that is based around monitoring GGT concentrations in rams after they are given a low dose of sporidesmin (<0.2-0.6mg/kg). Resistant animals show no to a mild increase in serum GGT activity. Sheep breeders who have used this service since its inception have increased the resistance of their flocks to sporidesmin by 4–5 fold as measured by resistance to a dose that is 4 to 5 times higher than the original dose at the beginning (Smith and Towers 2002).

Dairy industry studies since 1989 have established that resistance to FE is also inherited in cattle. Morris *et al.* (1990) studied 1523 heifers in 1989 and found a heritability of $\log_e$ GGT of 0.31 ± 0.1. This was lower than the 0.42 for FE susceptibility in sheep but the study design between the two studies was not directly comparable as Morris *et al.* (1990) determined the estimate from a natural challenge rather than sporidesmin dosing. Morris *et al.* (1998) studied 528 cattle that were either orally dosed with sporidesmin or grazed toxic pasture and found the univariate heritability estimates

for $\log_e$ GGT were 0.29 ± 0.15 in Friesians and 0.77 ± 0.13 in Jerseys. Livestock Improvement Corporation (LIC) only found heritabilities of 0.03-0.17 (Spelman R. J 2000), however this was determined by using a model whereby the outcome assessed was a subjective 5-point scale assessment from the farmer on the effects FE has had on their 2 and 3 year old sire proven daughters. The score ranged from not affected to dead from FE. This method is an extremely blunt and inaccurate way of testing for FE susceptibility and it is likely that this resulted in a considerable underestimation of FE heritability. Morris *et al.* (2002a) showed that heritability estimate of $\log_e$ GGT from 1500 FE-exposed cattle was 0.32.

Cullen N. G (2006) collected data from 572 specially reared sons (born in 2002-2004 and dosed with sporidesmin), and 3761 daughters in autumn 2004 to 2005 as well as combining this with data from 1173 animals born in 1986-1992). Heritability was estimated for Friesians (0.47 ± 0.07 for log GGT) and Jerseys (0.37 ± 0.06 log GGT). It was noted that heritability was sufficiently high that genetic progress would be made if selection was applied. In a subsequent analysis Cullen N.G (2011) compiled data from 14,799 cows from 66 herds over seven autumns and was able to take into accounts the levels of FE-protection provided by zinc sulphate administered through the water. This showed an estimate of $\log_e$ GGT of 0.34 ± 0.02.

Unfortunately, despite the high heritability of FE resistance demonstrated in dairy cattle, the dairy industry has only very recently adopted selection for FE resistance as a control measure for this disease.

## 2.5 EFFECTIVENESS OF MANAGEMENT OF FACIAL ECZEMA

The incidence of subclinical eczema is a good indicator of how well this disease is being managed by farmers. Towers (1978) determined the incidence of clinical and sub-clinical eczema from 10 Waikato dairy herds in May of 1976 by taking blood samples from 1357 cows and obtaining data from the farmers about clinical cases. This study showed that 9.4% of the cows tested displayed symptoms typical of facial eczema and 39% had abnormally high serum GGT activity (>30 IU/L). Five

percent of the cows had serum GGT activities > 1000IU/L, indicating severe recent liver damage. There was a wide variation in the incidence and severity of the liver damage. In June of 1984 a survey looking at the livers of 7948 cattle in a slaughter house in Northland found that 4.4% of cattle from farms in Northland showed evidence of FE affected livers and 2.8% from farms outside of Northland (Kearns 1985). At around the same time Faull (1986), in 100 randomly selected herds in the Palmerston North region, 11.2% of cattle had evidence of subclinical facial eczema (Faull 1986). During a serious outbreak of facial eczema in 1989, blood samples were obtained from 1593 first lactation heifers born in 1986 from 60 herds from Northland, Auckland and Taranaki. Overall, 29% of animals had elevated GGT values (>30IU/L) and 72% of herds had 2 or more animals with elevated GGT (Morris *et al.* 1990).

In May 1999, 350 North Island sire-proving-scheme farmers were surveyed by Livestock Improvement Corporation. Two thirds of farmers responded; of the responders 50% claimed to have had at least one case of FE. Overall, 11% of animals had FE, 5% were slightly affected, 5% were severely affected, 0.5% were culled due to FE and 0.5% died primarily from FE (Spelman R. J 2000).

In April and May 2008 it appeared that many dairy farmers in the Waikato were taken by surprise and many farmers suffered large clinical outbreaks and losses from sub-clinical facial eczema (Cuttance E, personal observations). It was clear that despite the large quantity of research on facial eczema and its prevention over the past 60 years, farmers were still having difficulty managing this disease effectively.

In 2011 DairyNZ and Sustainable Farming Fund invested in research initially aimed at trying to get a better understanding behind the natural spore count variability in paddocks, between paddocks and between farms and to try and find alternative ways of managing facial eczema without zinc. After this research was completed in 2013 it was deemed necessary to try and understand to what extent management of facial eczema was breaking down and possible reasons for these breakdowns. The end goal was to try and help farmers improve their management of this disease and reduce incidence of facial eczema. This thesis describes those studies.

# The effect of pasture sward mix on *Pithomyces Chartarum* spore counts in New Zealand

## 3.1 ABSTRACT

**Aim**

To compare pasture spore counts of *Pithomyces Chartarum* fungus on six sward type mixes to assess the benefit of sward type in the control of facial eczema.

**Method**

A set of 18 x 0.5 ha paddocks were set up at the DairyNZ research farm (Scott Farm) in Hamilton in March 2010 for separate research on the outcomes from sward mixes in paddocks. There were six sward mixes and three replicates of each. Low diversity meant that the paddock was sown with a single grass species and white clover. High diversity meant that the paddock was sown with a single grass species and white clover plus some prairie grass, chicory, plantain and lucerne.

Pasture samples were collected weekly from 31 January 2012 to 9 May 2012 from all 18 paddocks and pasture spore counted.

Statistical analysis comparing both high and low diversity and the different sward types were undertaken using generalized estimating equations to account for repeated measures of spore counts in paddocks over time.

**Results**

There was no difference in spore counts over time between high diversity and low diversity pastures (p=0.43). Both tall fescue paddocks (LDTF and HDTF) showed a lower spore count result than low diversity ryegrass over time (P=0.055 and 0.05; table 3.2)

**Conclusion**

The addition of chicory, plantain, lucerne and white clover into a ryegrass pasture did not provide any benefit into decreasing spore counts. Tall fescue paddocks showed lower spore counts over time than low diversity ryegrass.

## 3.2 INTRODUCTION

Facial eczema (FE) is a seasonal hepatogenous photosensitisation of ruminants caused by sporidesmin which is produced by the saprophytic fungus *Pithomyces chartarum* which grows on the dead and dying matter at the base of the pasture (Brook 1963). FE outbreaks occur when weather conditions are suitable for fungus growth and spore production.

The impact of different pasture species on the production of spores by *P. chartarum* and the subsequent risk of FE was evaluated using multiple paddocks in Northland, Waikato and Palmerston North from 1997-2000 (Keogh 2001). Chicory (*Cichorium intybus*)**,** clover *(Trifolium pratense and Trifolium repens)* and tall fescue *(Festuca arundinacea)* based pastures were compared to ryegrass-dominant pastures *(Lolium perenne)* by assessing faecal spore counts alongside serum GGT activity and liver damage recorded at slaughter in cattle grazing the experimental paddocks. Results from these studies suggested chicory, red and white clover and tall fescue support low levels of *P. chartarum* while ryegrass dominant pastures do not. However, the faecal spore count data were taken from a number of different paddocks in 3 different geographical locations in New Zealand over three different years without any statistical analysis.

In addition to the faecal spore count study, leaves of ryegrass, cocksfoot *(Dactylis glomerata),* browntop *(Agrostis tenuis),* Yorkshire fog *(Holcus lanatus),* chicory, red clover, white clover, lotus *(Lotus pedunculatus)* and tall fescue were inserted into the base of plots growing these species in autumn (March) 1997. Leaves were sequentially harvested on six occasions during the following 8 week period and *P. chartarum* spore loads determined (Keogh 2001).

Results from these studies suggested chicory, red and white clover, lotus and tall fescue support low levels of *P. chartarum* while ryegrass, cocksfoot, browntop, and Yorkshire fog supported high levels of *P. chartarum*. However, spore load results from inserted leaves had few measurements to compare as after harvest many of the inserted leaves had decomposed or been removed by earthworms.

The aim of this study was to build on the previous data by comparing naturally occurring spore counts on six sward mixes in a controlled environment in one location.

## 3.3 MATERIALS AND METHOD

A set of 18 x 0.5 ha paddocks were set up at the DairyNZ research farm (Scott Farm) in Hamilton in March 2010 for separate research looking at milk production outcomes from cattle grazing different sward mixes. Six different types of sward mixes were used and each sward had three paddocks sown with that mix, giving 18 total paddocks (Table 3.1).

**Table 3.1: Sward mixes for research paddocks.**

| Treatment group | Treatment code | Treatment details |
|---|---|---|
| 1 | HDHS | High diversity, high sugar ryegrass |
| 2 | HDRG | High diversity, ryegrass |
| 3 | HDTF | High diversity, tall fescue |
| 4 | LDHS | Low diversity, high sugar ryegrass |
| 5 | LDRG | Low diversity, ryegrass |
| 6 | LDTF | Low diversity, tall fescue |

The six different sward mixes could be grouped into either low or high diversity mixes. Low diversity meant that the paddock was sown with a single grass species (perennial ryegrass, high sugar

perennial ryegrass or tall fescue) in addition to white clover. High diversity meant that the paddock was sown with one of the main grass species as described above and white clover but also had prairie grass, chicory, plantain and Lucerne sown in.

Spore counts were taken weekly from each of the 18 paddocks starting from 31/1/12. Pasture samples for each section were taken by walking in a diagonal line from the corner of a section to the opposite corner and collecting 20 g of pasture at 10 locations along the way by cutting the pasture at the base of the sward. The sampler collected whatever was at their feet for the samples regardless of whether it was grass, chicory or lucerne. The final sample was mixed thoroughly in the bag before choosing the sample to spore count from.

***Spore counting***

The technique used was a modified technique of that described by Di Menna and Bailey (1973), whereby 200 g of pasture that comprised each sample was gently mixed inside the collection bag. Sixty grams of grass was selected from the 200 g and added to 600 mL of water in a plastic container. The container was shaken vigorously for 3 minutes. The pasture was then removed from the container and an eye dropper used to retrieve a sample of the wash water while agitating the container back and forth. A cover slip was applied over the grids of a haemocytometer slide and both sides of the slide filled with the sample of wash water. Spores were counted using a microscope at 100× magnification. The total pasture spore count/g pasture was estimated by multiplying the number of observed spores by 10,000.

***Statistical analyses***

Each spore count from each paddock on an individual date was visualised by plotting the results as a dot plot. Using R for Windows (Version 3.1.2, https://www.r-project.org/) a non-parametric smoothed line of best fit and the uncertainty around the line of best fit was calculated and superimposed over the dot plot.

All analyses were undertaken using generalised estimating equations (Hardin 2005) to account for repeated measures of spore counts in paddocks over time. An auto-regressive correlation structure was used to account for spore counts being correlated over time with the correlation depending linearly on its own previous values.

Two models were used; the first included time and diversity of sward mix as fixed effects, while the second included time and sward mix as fixed effects.

Data analyses were conducted using the statistical package R version 3.1.2 (R Development Core Team 2014l R Foundation for Statistical Computing, Vienna, Austria).

## 3.4 RESULTS

The trend in spore counts was similar throughout the study period for both high and low diversity (Figure 3.1). Spore counts rose in February and then decreased throughout March. There was no difference in spore counts over time between high diversity and low diversity pastures (p=0.43).

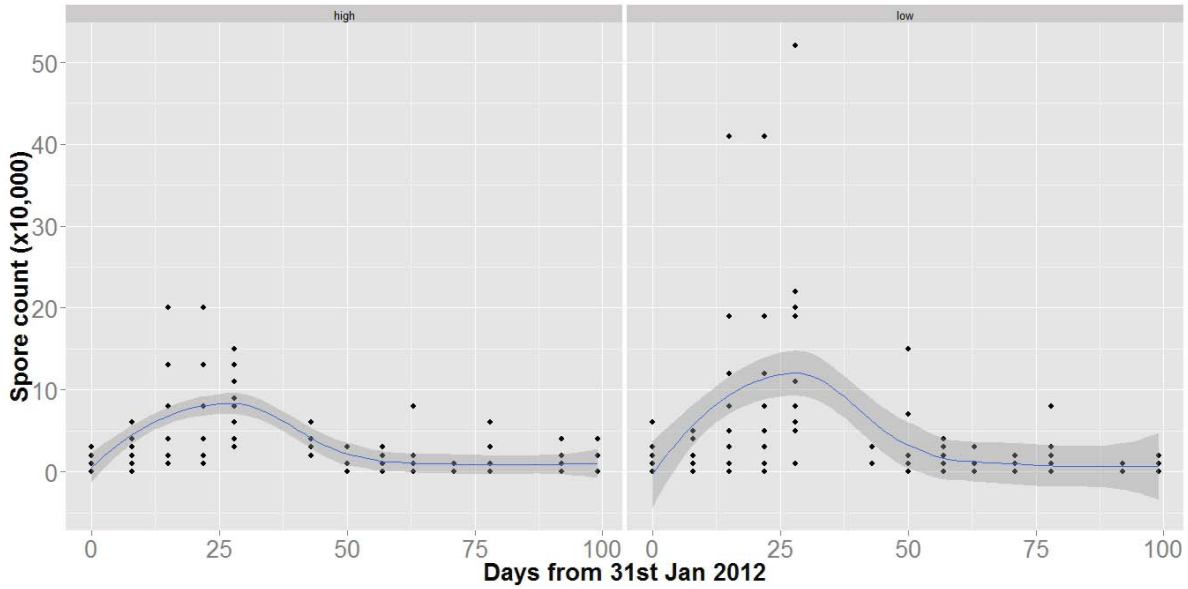


*Figure 3.1: Plot of spore counts in the high and low diversity paddocks over time. The blue line superimposed on each plot is a (non-parametric) smoothed line of best fit; the shaded areas indicate the uncertainty around the line of best fit.*

The pattern for individual sward mixes is shown in Figure 3.2. The majority of spore counts were less than 100,000/g, with most paddocks having spore counts below 20,000 spores/g for the majority of

the study period. Low diversity ryegrass (LDRG), low diversity high sugar (LDHS) and high diversity high sugar (HDHS) had small spore count peaks in March (up to 50,000 spores/g), while the tall fescue paddocks (LDTF and HDTF) were consistently low (<20,000 spores/g). Both tall fescue paddocks (LDTF and HDTF) showed a lower spore count result than low diversity ryegrass over time (P=0.055 and 0.05; table 3.2)

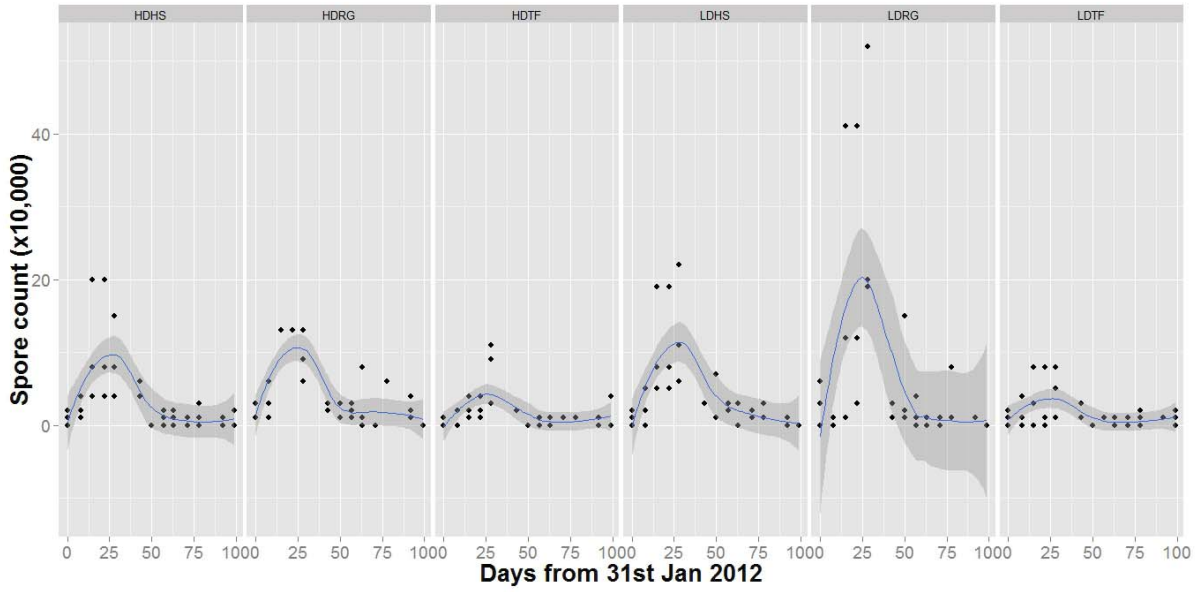


*Figure 3.2: Comparison of spore counts between the 6 different diversity treatments. The blue line superimposed on each plot is a (non-parametric) smoothed line of best fit; the shaded areas indicate the uncertainty around the line of best fit.*

**Table 3.2. Multivariable linear model for the effect of six different types of pastures spore counts over time (weeks)**

| Variable | Estimate (SE) | P value |
|---|---|---|
| Weeks after treatment | -0.36 (0.08) | <0.001 |
| Low Diversity Ryegrass | *Reference* | |
| High Diversity Ryegrass | -2.1 (2.2) | 0.33 |
| Low Diversity Tall Fescue | -4.4 (2.22)[a] | 0.05 |
| High Diversity Tall Fescue | -4.3 (2.34) | 0.055 |
| Low Diversity High Sugar | -2.4 (2.34) | 0.30 |
| High Diversity High Sugar | -2.8 (2.31) | 0.23 |

a. Interpretation: In comparison to low diversity ryegrass, low diversity tall fescue spore counts were 44,000 spores/g pasture less per week.

## 3.5 DISCUSSION

The results of this study show that there was a difference in spore counts between low diversity ryegrass and swards containing tall fescue. The study also suggested that adding in diversity through use of herbs did not decrease spore counts.

The results shown for tall fescue are similar to that of Keogh (1998) who reported that tall fescue supported lower spore counts than ryegrass. This may be because tall fescue has a higher branching structure, more resistance to microbial degradation of old stems, a lower tillering rate and more longevity of leaves and tillers than perennial ryegrass (Hume and Brock 1997), thus reducing the quantity of available dead matter at the base of the sward for the fungus to live on.

The effect found in this study may have been more pronounced with higher spore counts. The majority of spore counts were between 0 and 20,000 spores/g of pasture with the highest count only reaching 50,000 spores/g of pasture. In contrast, Keogh (1998) had spore counts up to 1,000,000 spores/g faecal matter, more than 10 times his recommended intervention level.

Chicory and red and white clovers on their own have also been shown to support lower spore counts than ryegrass (Keogh 1998). However, for these mixes if there is ingression of ryegrass into the pasture mix there will be a breakdown in facial eczema control (Keogh 1998). The results of the current study support this conclusion as we found no benefit of adding chicory, plantain, lucerne and white clover to ryegrass. In order for pastures to be 'safe' for FE, they need to contain no ryegrass.

## 3.6 CONCLUSION

This study demonstrated firstly that the addition of chicory, plantain, lucerne and white clover into a ryegrass pasture did not provide any benefit in terms of decreasing spore counts and that tall fescue

swards either mixed in with other herbs or as a pure sward supported lower spore counts than a pure ryegrass sward.

# The influence of lime and nitrogen fertilizers on spore counts of *Pithomyces chartarum* in pasture

## 4.1 ABSTRACT

**Aims:**

To determine whether the application of lime or nitrogen to pasture significantly affects the spore counts of *Pithomyces chartarum*.

**Materials and methods:**

***Lime application***

The lime application studies were undertaken on a spring-calving, pasture-based commercial dairy farm in Te Awamutu, New Zealand. Two different application timings were tested: 1) application prior to the risk period (pre-summer lime); and 2) application in response to elevated spore counts (autumn lime).

The pre-summer lime application was undertaken on 6th November 2012. Five paddocks were split into three equal sections. In two of the sections, lime was applied at either 1.5 t/ha or 2.5 t/ha; the central section was left as an untreated control. Each section was sampled for spore counting weekly from the 16 January to 15 May 2013.

The autumn lime application occurred when the average spore counts in 3/5 of the nominated paddocks became > 100,000 spores/g of pasture (20th March 2013). The affected paddocks were then divided into three equal sections and sampled for spore counting. Immediately after spore

counting, lime was applied as described above and spore counting for each section continued weekly until 15 May 2013.

***Nitrogen application***

This study was undertaken on three commercial dairy farms in Te Awamutu, New Zealand using two randomly selected paddocks on each farm. Selected paddocks were divided into three equal sections and, on 20 December 2012, nitrogen in the form of urea was applied at either 50 or 80 kg urea/ha to two of the sections; the central section remained as an untreated control. Weekly spore counting for each individual section started on 16 January 2013 and stopped on 15 May 2013.

**Results**

For all studies and treatments there was no difference between spore counts or change in spore counts between treated and control sections of the paddocks.

**Conclusion**

This study found that application of lime before the risk period for facial eczema (in November), application of lime after a spore count rise, (in March) or urea application (in December) did not affect the number of spores produced by *Pithomyces chartarum*.

**Clinical relevance**

This study does not support previous suggestions that fertilising pasture with lime or urea could alter the spore counts of *P. chartarum*. Fertiliser use does not provide an alternative to, or support, conventional methods of FE control such as zinc prophylaxis or treatment of pasture with fungicides.

## 4.2 INTRODUCTION

Facial eczema (FE) is a seasonal hepatogenous photosensitisation of camelids and ruminants caused by grazing pasture containing spores of *Pithomyces chartarum* (Brook 1963). As *P. chartarum* sporulates it produces the epidithiodioxopiperazine toxin, sporidesmin, which, when ingested by

susceptible grazing animals, causes inflammation and blockage of the bile ducts. Photosensitising pigments that are normally excreted in the bile accumulate in the circulating blood and when animals are exposed to sunlight, causes lesions on unpigmented skin (Di Menna *et al.* 1970).

Losses from FE arise from deaths, condemnation of carcasses and impaired productivity including loss of live weight, reproductive failure and decreased milk production. In dairy cattle FE can cause significant production losses for dairy farmers. Towers and Smith (1978) demonstrated a 25% drop in daily milk volume when cows were artificially dosed with sporidesmin, while Smith and Embling (1983) showed a reduction of 10% in milk volume when cows were naturally exposed to long periods of low spore counts (0-90,000 spores/g) on pasture.

Early attempts at FE control in New Zealand focused on avoiding exposure to pasture known to have high pasture spore counts (Brook, 1963; McMeekan, 1958; Smith, 1987). Since then most of the focus has been on the development of fungicides (Sinclair and Howe 1968; Wallace 1976; Parle and Di Menna 1972) to kill *P. chartarum* on pasture (and thus reduce the production of sporidesmin) and the use of zinc at high doses to protect against the toxic effects of ingested sporidesmin (Towers and Smith 1978; Smith and Embling 1983; Munday et al. 2001). Used correctly, zinc products and fungicides are effective at reducing the impact of FE, however they are time consuming to administer and can be expensive.

***Lime***

The application of lime fertilisers on pastures as a protective measure against facial eczema has been debated for more than 75 years (Anonymous, 1970). Lime fertilizers are routinely applied to agricultural soils in New Zealand to raise pH into the range of 5.7-6.5 which is considered optimal for pasture growth (Haynes and Naidu 1998). The anecdotal belief from many farmers is that the application of lime on pastures helps prevent the growth of *P. chartarum* fungus, and/or that it helps decrease pasture spore counts, if they are present. The theory proposed is that the change in pH of the soil may alter the growth and sporulation of the *P. chartarum* fungus as soil pH can influence

biomass composition of fungi and bacteria (Bardgett et al. 2001; Fierer and Jackson 2006; Rousk et al. 2009).

A widely disseminated research paper (Grierson 2007) concluded that the application of 2.5t/ha of lime caused a reduction in pasture spore counts 7-14 days after application. The lime was applied to one 20 x 5m plot on a Katikati deer farm and the spore counts compared to those from an adjoining untreated control plot. Pasture sampling was then carried out weekly/fortnightly for two months starting 7 days after application of lime. This was carried out for 3 years, starting in 2005. In each year, lime was applied on a different plot at approximately similar dates to previous years. In addition, in 2007, spore counts were collected from the previously treated plots and compared to the control plot for that year to identify if there were residual effects of the application of lime. Grierson concluded that lime application reduced spore counts within 7 days of application and that this effect lasted for up to 2 years.

However, as the initial pasture spore counts for the treatment and control plots were not recorded before the application of lime, it is quite possible that as there was only one control and one treated plot each year, the differences between treated and control plots were due to pre-existing differences in spore counts rather than treatment, especially since the same control plot was used over the 3 years of the study. To the best of our knowledge there have been no other controlled studies investigating the effects of lime on pasture spore counts.

***Nitrogen***

New Zealand dairy farmers depend on nitrogen fixation by white clover as the main source of nitrogen (N) input into their pastures (Ledgard *et al.* 1996). Many herd managers routinely apply moderate amounts of fertiliser N (between 50 and 200kg N/ha/yr) to pasture, mainly in the form of urea.

Nitrogen has effects on the growth of ryegrass and its survival in dry conditions. An observational study by (Keogh 1973, 1975) showed that facial eczema spore counts tended to be higher in urine patches. The high N composition of these patches (Keogh 1979) supports densely tillered, rapidly

growing ryegrass. If mature leaf and leaf sheath is not removed by previous grazing this rapidly growing grass could accumulate more dead and dying matter at the base and could therefore provide favourable conditions for fungus development. In addition, Lucanus *et al.* (1960) suggested that nitrogen application reduced the ability of ryegrass to survive high temperature, low moisture conditions which are typical of New Zealand summers. An increase in the amount of dead ryegrass within the pasture sward is thought to provide favourable conditions for fungus development (Brook 1963).

Similar to the paucity of studies investigating the effect of lime on pasture spore counts, we know of no studies that have specifically investigated the impact of nitrogen application on pasture spore counts.

The aims of this study were to determine if: a) lime application in November, prior to the risk season for FE decreased subsequent *Pithomyces chartarum* spore counts; b) lime application on high spore count pasture in the Autumn decreased *Pithomyces chartarum* spore counts; and c) if nitrogen application on pasture in December increased *Pithomyces chartarum* spore counts.

## 4.3 MATERIALS AND METHODS

***Pre-summer lime study***

This study was carried out on a single spring-calving, 500 cow pasture-based commercial dairy farm in Te Awamutu, Waikato, New Zealand.

At the start of the study in October 2012, the participating herd manager was asked to provide a sketch map of the 65 paddocks that comprised the 125 ha farm. Each paddock was numbered from 1 to 65. Five numbers were selected from the list at random and each paddock divided into three equal sections. Each section within each paddock was labelled on a farm map as A, B and C. The vertices of each section were identified using 50 cm × 5 cm × 5 cm pegs.

On 29 October 2012 each of the three sections within the 5 paddocks were soil tested by an experienced technician from a local fertiliser company (Ravensdown Fertilizer New Zealand Ltd). The

diagonal of each section was traversed by the technician and a total of ten soil samples retrieved, pooled and submitted to Analytical Research Laboratories Ltd, Hamilton, where they were tested for soil pH.

On 6 November 2012 lime was applied by a contractor at 1.5 t/ha on section A and 2.5 t/ha on section C. Section B, which was in the middle, was left as an untreated control. The application of lime was overseen by the author. The paddocks were approximately 2 ha in size, so each trial section was about 0.66 ha. The day was calm so there was minimal drift of light lime dust to other sections. The pasture cover of the paddocks on the day of lime application was estimated by eye by the farmer as between 1500kgDM/ha and 2200kgDM/ha.

Spore counting was carried out weekly by a technician (who remained the same throughout the trial) from16 January to 15 May 2013. Pasture samples within each section were retrieved by walking in a diagonal line from the corner of a section to the opposite corner and collecting approximately 20 g of pasture at 10 locations along the way by cutting the pasture at the base of the sward.

***Autumn lime study***

This study was carried out on the same commercial dairy farm that participated in the pre-summer lime application trial. Eligible paddocks for this study were those that were not involved in the pre-summer lime application study. Each paddock was numbered from 1 to 60 and five numbers were randomly selected from the list of 60.

From 16 January 2013, spore counts were determined using the same method described for the pre-summer lime application trial until the average spore count was greater than 100,000 spores/g of pasture for a paddock. On 20$^{th}$ March 2013 3/5 paddocks being tested had counts over 100,000 spores/g of pasture. On 22 March 2013, each of these three paddocks were spilt into three equal sections by pacing out the length. Each section was labelled on a farm map as A, B or C. The vertices of each section were identified using 50 cm × 5 cm × 5 cm pegs. Each of the sections of the paddock had spore counts determined by the same method described above. Immediately after the spore

count on the 22 March 2013, for each section of the three different paddocks with spore counts over 100,000 spores/g pasture, lime was applied by a contractor at 1.5t/ha on section A and 2.5t/ha on section C. Section B was left as an untreated control. Pasture cover as estimated by eye by the farmer was approximately 1800-2000kgDM/ha. Spore counting for each section continued weekly until 15 May 2013.

The other two remaining eligible paddocks continued to be tested until 15 May 2013 but at no point did they have spore counts over 100,000 spores/g.

***Nitrogen study***

This study was carried out on three commercial dairy farms in the Waikato region of New Zealand. The three farms were well established dairy farms that milked approximately 400, 600 and 700 cows on 150, 200 and 200 ha, respectively.

On each farm two paddocks were selected at random using the approach described for the pre-summer and autumn lime studies. Selected paddocks (approximate area 1.5-2 ha) were divided into three equal sections and labelled on a farm map as A, B and C. The edges of each section were identified using 50 cm × 5 cm × 5 cm pegs.

On 20 December 2012 nitrogen, in the form of urea, was applied to each of the selected paddocks of each of the farms by a contractor at 50 kg urea/ha on section A and 80 kg urea/ha on section C. Section B which was in the middle was left as an untreated control. Application of urea was overseen by the author. Pasture cover as estimated by the farmers was between 1500kgDM and 2500kgDM/ha. Pasture cover was not measured with a plate meter prior to application. Spore counting was undertaken weekly from 16th January to 15th May 2013. Pasture samples for each section were collected in the same manner as described in the lime study.

***Spore counting***

All spore counting was undertaken by two technicians who remained the same throughout the trial. The technique used was a modified technique of that described by Di Menna and Bailey (1973),

whereby the 200 g of pasture that comprised each sample was gently mixed inside the collection bag. Sixty grams of grass was selected from the 200 g and added to 600 mL of water in a plastic container. The container was shaken vigorously for 3 minutes. The pasture was then removed from the container and an eye dropper used to retrieve a sample of water mixture while agitating the container back and forth. A cover slip was applied over the grids of a haemocytometer slide and both chambers of the slide were filled with the sample solution. Spores were counted using a microscope at 100× magnification. The total pasture spore count/g pasture was estimated by multiplying the number of observed spores by 10,000.

**Statistical analyses**

For all three studies, the median of all the spore counts for each treatment were plotted against time.

For the pre-summer lime application and nitrogen study, the estimated spore count was compared to treatment application rate and days since the commencement of spore counting.

For the autumn lime application, a difference in the reduction of spore counts from the previous count starting at the initial spore counts prior to lime application was compared to lime application rate and days since the application of lime.

All analyses were undertaken using generalised estimating equations to account for repeated measures of spore counts in paddocks over time. An auto-regressive correlation structure was used to account for spore counts being correlated over time with the correlation depending linearly on its own previous values.

As the trial comparing nitrogen application rates was carried out on different farms, farm was also added as a fixed effect. All data analyses were conducted using the statistical package R version 3.1.2 (R Development Core Team 2014l R Foundation for Statistical Computing, Vienna, Austria).

## 4.4 RESULTS

***Pre-summer lime application***

Three out of the five study paddocks had pH results classed as low; between 5.5-5.8. The remaining two had pH results classed as optimum to high; between 6.0-6.2.

For all treatment groups there was an increase in pasture spore counts at 120 days (6 March 2013) after lime application. Spore counts decreased in all treatment groups 160 days after treatment (15$^{t}$ April 2013) (Figure 4.1). In comparison to the control, treatment with lime pre-summer at 1.5t/ha (p=0.75) and at 2.5t/ha (p=0.26) did not significantly affect spore counts over time.

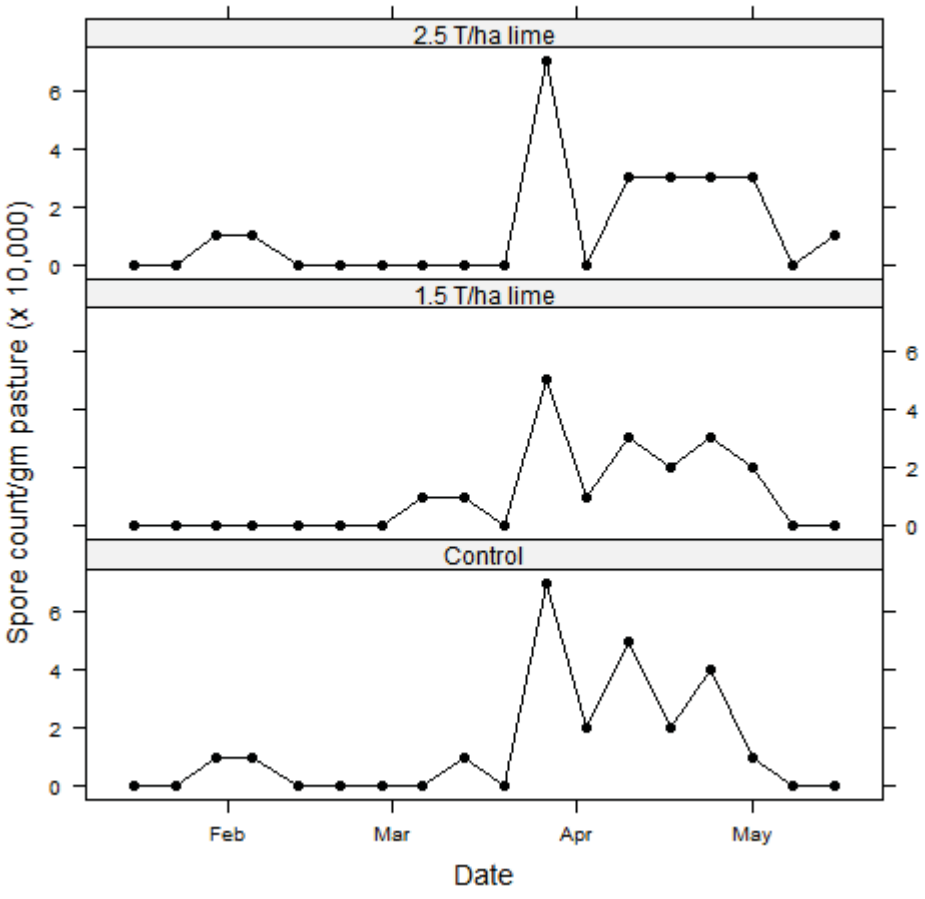


*Figure 4.1: Pre-summer lime trial study. Line plots showing the median pasture spore counts (× 10,000) per gram of pasture for each treatment (5 plots/treatment) over time following the study start date (16$^{th}$ January 2013).*

***Autumn lime application***

For all treatment groups there was an initial decrease in spore count. Between day 20-30 most of the spore counts increased and then decreased again by day 35 (Figure 4.2). In comparison to the control, treatment with lime on spore counts over 100,000 spores/g pasture at 1.5t/ha (p=0.41) and at 2.5t/ha (p=0.75) did not significantly affect spore counts over time.

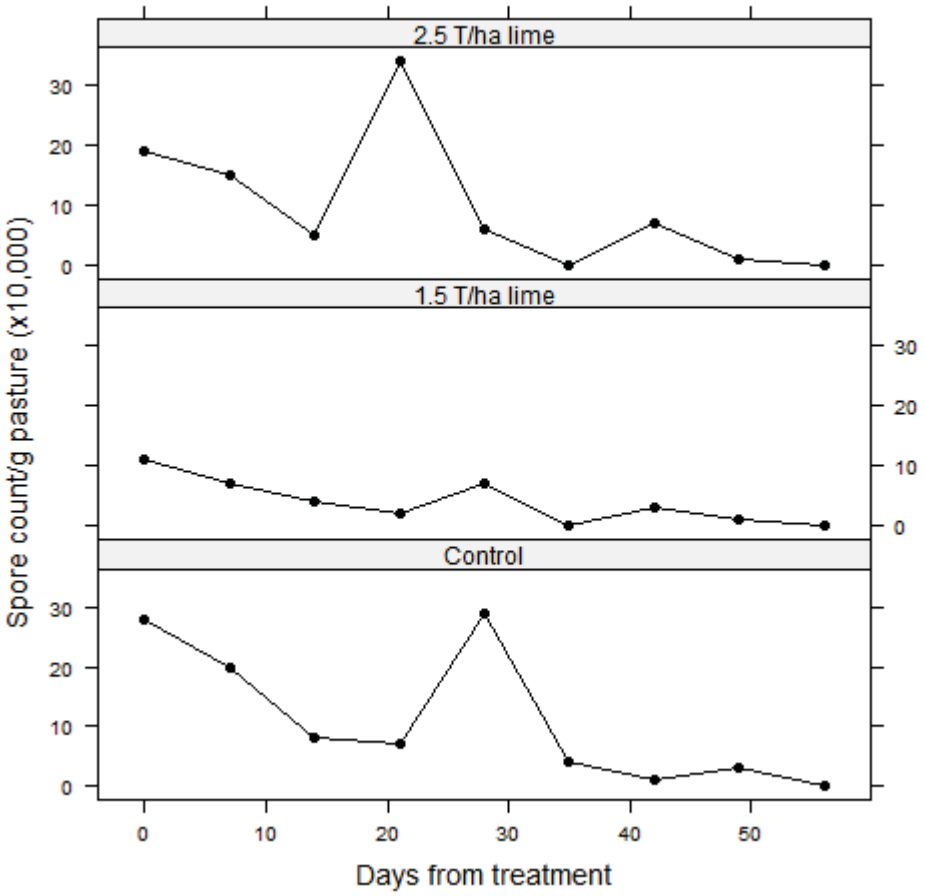


*Figure 4.2: Autumn lime trial study. Line plots showing median pasture spore counts (× 10,000) per gram of pasture for each treatment (5 plots/treatment) as a function of the number of days following study start where lime was applied (22nd March 2013).*

***Nitrogen application***

For all treatment groups the spore counts remained low throughout the trial period with only two counts above 30,000 spores/g. The median spore count for each treatment remained below 20,000 throughout the trial period (Figure 4.3). In comparison to the control, treatment with nitrogen at 50kg/ha (p=0.90) and at 80kg/ha (p=0.49) did not alter spore counts over time.

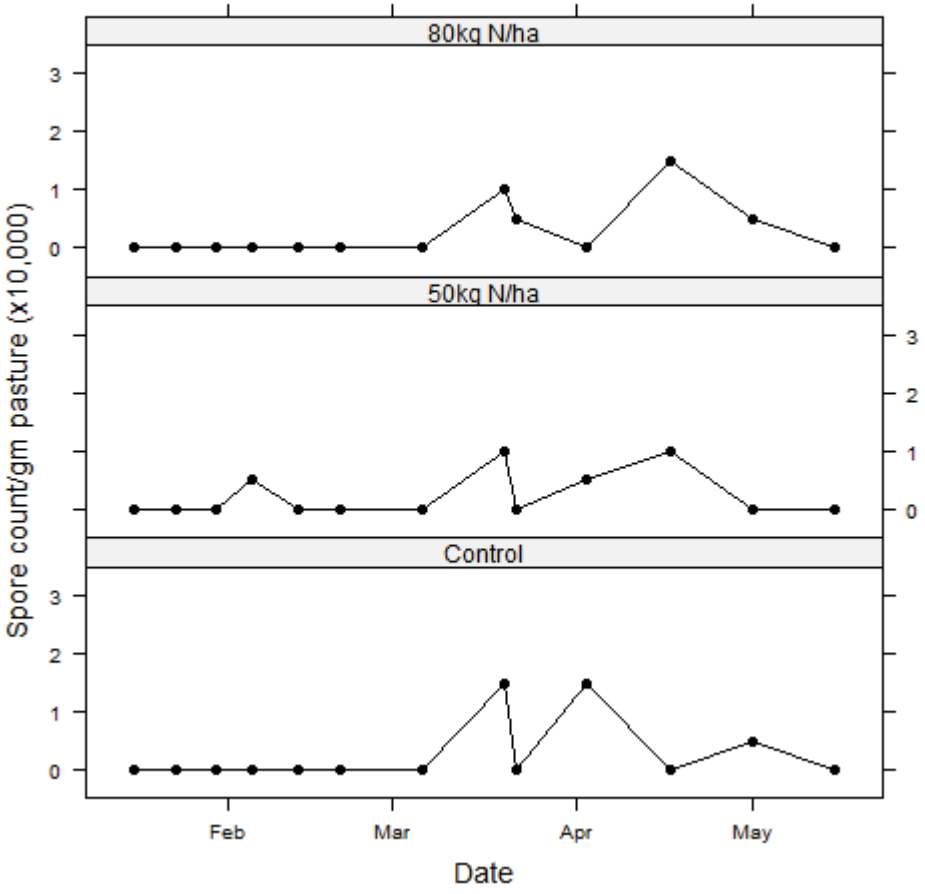


*Figure 4.3: Nitrogen study. Line plots showing median pasture spore counts (× 10,000) per gram of pasture for each treatment (6 plots/treatment) over time following the study start date (16th January 2013).*

## 4.5 DISCUSSION

We identified no association between the use of lime and pasture spore counts when lime was applied either before summer or during autumn. Our findings differ from those of Grierson (2007)

who observed that spore counts on a plot treated with lime in March/April were on average ~20% of those in an adjoining untreated plot and different to many of the anecdotal suggestions from farmers. However, Grierson (2007) did not account for spore count prior to treatment and only included one treatment and one control plot per year. This study accounted for spore count in the autumn application and used multiple divided paddocks resulting in five control and 10 treatment plots. However, in contrast to this study which evaluated the effect of lime over one year only, Grierson (2007) evaluated the effect of lime application in three separate years. He found the same effect in each of the three years, but this still means that he had only three treatment plots and, because the same plot was used each year, only one control plot. Without information on pre-treatment spore counts, the small number of treatment plots means that the differences seen could be simply due to the treatment plots having, by chance, inherently lower spore counts (due to different microclimates). This is exacerbated by having only one control plot, which as the three years of data show was a paddock which had a microclimate which was favourable for the growth of *P. chartarum.*

In addition, Grierson (2007) reported that the effect of lime treatment persisted for up to two years after treatment, with plots treated in 2005 and 2006 having lower spore counts than the control plot in 2007. This postulates a lime effect which is effective within 7 days and which persists for up to two years. It is more feasible that the differences in 2007 were due to systematic biases between plots in their inherent suitability for *P. chartarum.*

It is possible that the lack of spore count difference observed in this study could have related to the starting pH of the plots where it was applied. Rousk *et al.* 2009 showed that fungal populations in soil increased when the soil pH became more acidic from 8.3 down to a pH of 4.5. The effect of soil pH on *P. chartarum* growth is not known, but the data from Rousk *et al.* 2009 suggest that it is possible that a response in the growth of *P.chartarum* may only be observed if the initial soil pH is highly acidic. However, three out of the five paddocks were classed below optimum pH of 5.8-6.0 and no paddock effect was observed. Further controlled studies are required to establish the impact of pH on *P. chartarum* growth.

We also found that the application of urea at 50 kg/ha or 80 kg/ha in December had no significant effect on spore counts during the facial eczema season. As the grass growth response to nitrogen is largely dependent on the temperature of the soil and its moisture content, it is possible that these results could change depending on the season. An abnormally dry season or a season particularly suited to excellent pasture growth could alter the amount of dead matter at the base of the sward and therefore give different results. It is likely that the effect nitrogen could have on pasture spore counts would be influenced more by individual management of pasture rather than the nitrogen application itself.

As the response of the pasture (and thus, potentially, the spore count) to lime and nitrogen (particularly the latter) is extremely weather dependent (Bircham and Crouchley 1976; Wheeler 1998; Zhang and Tillman 2007) one year's data may not be representative of the range of potential response. Further research, under different weather conditions and across more of New Zealand would be useful to confirm our findings. Such studies should, in particular focus on whether the response of the pasture to N or lime alters the response of spore count to those products.

## 4.6 CONCLUSION

Application of lime either before the FE risk season or on pastures with high spore counts had no effect on pasture spore counts. Similarly, application of nitrogen in the form of urea in December did not have any effect on pasture spore counts.

# Variability of *Pithomyces Chartarum* spore counting

## 5.1 ABSTRACT

**Aims:**

To quantify the variability of spore counts within a paddock, within a grass sample and between sample aliquots and to identify whether that variability could be attributed to the composition of those samples.

**Materials and Methods:**

In four commercial dairy farms in the Waikato region of New Zealand, a single paddock was selected for grass sampling. Forty sampling points were defined using pegs within the paddock boundary. Each participant farm was visited by rural technicians once weekly for 19 weeks starting on the 7th January 2013 to measure height of the pasture and to collect a 60-200g pasture sample along a 1m radius line crossing the peg. On the same date a paddock sample was taken by walking from one corner of the paddock to the other and taking 10 x 20 gram samples at even intervals along the diagonal.

For each 200 g pasture sample collected, three separate 60 g samples were spore counted with the wash technique described by Oldman and Di Menna (1983b), and for each 60 g pasture sample, 10 water aliquots were counted. A 60 g sample of pasture from each peg site was selected for composition analysis (dry matter, proportion of green, yellow and dead grass and clover).

***Statistical analysis***

The agreement between 1, 2 or 3 aliquots per sample was assessed using predictions based on a generalised linear repeat measures model (with a Poisson distribution and a log link). The predictions were compared using limits-of-agreement analysis. The relationship of spore counts and individual grass components was assessed with a negative binomial model with random effects for repeated measurements taken at peg sites. The final model was built using a backwards selection of variables.

**Results**

This study has identified that the repeatability of spore counting is poor, either when comparing different 60 g grass samples selected from a combined 200 g grass sample or comparing between multiple aliquots selected from the same wash water.

The multivariable analysis of composition showed that increased height of pasture was associated with a lower spore count and a medium dry matter estimation was associated with a higher spore count than wet grass.

**Conclusion**

If spore counts are to be used for monitoring purposes to identify when to start and finish FE prevention programmes, we recommend that at least three aliquots per wash water are selected and that for stopping programmes at least 3 low spore counts must be recorded.

**Clinical relevance**

There has not been a known validation of the current spore counting technique universally adopted amongst vets and farmers. This study validates the current technique and provides some practical expectations of the test for farmers.

## 5.2 INTRODUCTION

Facial eczema (FE) is a seasonal hepatogenous photosensitisation of ruminants and camelids caused by the ingestion of fungal spores containing the hepatotoxin sporidesmin. The resulting liver damage means that photosensitising pigments that are normally excreted in the bile accumulate in the circulating blood and, when the animals are exposed to sunlight, cause photosensitisation particularly on unpigmented skin (Di Menna *et al.* 1970).

Losses from FE arise from deaths, condemnation of carcasses and a reduction in production parameters including live weight, fertility and milk yield. Production losses due to FE in New Zealand, adjusting for inflation, may range up to $215 million NZ$ for sheep and beef cattle alone[1] (Anonymous 1990).

In dairy cattle FE can cause significant production losses for dairy farmers. Towers and Smith (1978) showed a drop in milk volume of up to 25% when cows were artificially dosed with sporidesmin, while Smith and Embling (1983) showed a reduction of 10% in milk volume when cows were naturally exposed to long periods of low spore counts (0-90,000 spores/g) on pasture.

The spores containing the sporidesmin are produced by the saprophytic fungus *Pithomyces chartarum* which grows on the dead and dying matter at the base of the pasture sward (Brook 1963). The majority of spores are found below the mid- height of the sward but they can also be found on green growing leaves (Brook 1963) as they will blow in the wind especially when the ground is disturbed and can adhere to growing grass and clover (Thornton and Sinclair 1960).

Optimal growth of the fungus is considered to occur at 24 °C and sporulation at 100% humidity; under these conditions germination of the fungus can occur within 30 minutes (Smith and Crawley 1962) and sporulation within 2 days (Ross 1962). Spores can be found in the pasture all year around but only in low numbers when conditions are not suitable.

[1] Calculated using the calculator at www.rbnz.govt.nz/statistics/0135595.html, (accessed 30/06/2015)

Spore counting is currently the most widely used method of assessing the potential intake of toxic spores by grazing animals and thus their risk of facial eczema. Counting spores is not a direct measure of pasture toxicity, but in vitro studies have shown that the amount of toxin produced is proportional to the number of spores in the culture (Di Menna and Bailey 1973; Collin *et al.* 1995), and direct measurement of sporidesmin concentrations is currently not feasible in a timely manner and in a general veterinary practice setting.

Thornton and Sinclair (1960) were the first known researchers to develop a "wash technique" for assessing spore numbers. Twenty-five grams of fresh grass, cut at a height of 2.5 cm with hand box shears, was added to tap water (250 mL plus 1 drop of a sodium dodecyl benzenesulfonate-based detergent ['Teepol']) and shaken vigorously by hand for 1 minute in a jar. The contents were then strained through a 2 mm sieve. Counts of spores were made on the washings with the use of a Neubauer counting chamber. In each chamber the spores in five 1 $mm^2$ grid squares (centre and four corners) were counted.

The statistical analysis of this technique was limited; the authors reported that its accuracy and reproducibility were good, but no details of the analysis used or its outcome were reported. However, they did report that there was a reasonable correlation (r =0.645) between their spore counts and the then current test for sporidesmin (the 'beaker' test).

This initial technique has been modified over time. Di Menna and Bailey (1973) used 25 g samples of well-mixed pasture leaves, and mixed them further, using a mechanical shaker, with 250 mL of water for 5 minutes. The wash water was then placed, without preliminary straining, onto haemocytometer slides and the spores in 2 $mm^3$ counted. Each spore seen was stated as representing 5 000 spores/g of leaf (wet weight). They reported that the mean difference between counts of the same wash water (n=7 samples) was 9 000 spores/g (range 0—15 000). They also compared the results from 12 grass samples where two separate preparations were made from each sample. In this case the mean difference was 8 000 spores/g (range 0—25 000).

In that study, Di Menna and Bailey (1973) reported that in the last 2 years of the 4-year study period, they did not include 'old, partially disrupted spores' in the spore count on the basis that they were 'probably non-toxic'. There was no discussion in that paper of how this diagnosis of non-toxic spores was made, the proportion of spores which were non-toxic, or whether the reliability testing disregarded such spores. Subsequent studies have not reported excluding any spores on the basis of their appearance and it is not part of the standard protocol for spore testing.

Collin *et al.* (1995) used samples of pasture collected from 5 sites in a paddock cut at 1 cm above the ground. Samples were mixed and cut to 4 cm length then 15 g of forage was placed in a plastic bag with 150 mL of tap water and squeezed every few seconds for a minute. An aliquot of this water was then put under a microscope in a counting grid. Collin *et al.* (1995) compared the relationship between spore counts and sporidesmin concentrations (determined using an enzyme immunoassay) in pasture using samples taken from eight paddocks, twice a week for a period of 4 months from February to May 1994. They reported that there was a significant linear relationship between sporidesmin concentration and spore count ($r = 0.66$).

The spore counting technique recommended by Oldman and Di Menna (1983) is the standard method which is currently used by the majority of veterinarians, farmers, laboratories and researchers. In this method, 200 g of pasture is collected by walking diagonally across a paddock and stopping 10 times to collect approximately 20 g cut at ground level. These samples are then gently mixed inside the collection bag, and 60 g of pasture then removed. This is then added to 600 mL of water in a plastic container. The container is then shaken vigorously for 3 minutes, the pasture removed, and an eye dropper used to collect a sample of the solution while gently agitating the container back and forth. A cover slip is then applied over the grids of the haematocytometer and both sides of the slide are filled with the sample solution. Spores are counted using a microscope at 100x magnification. Depending on the depth of the grids the total pasture spore counts/g pasture are estimated by multiplying the number of observed spores by 5 000 or 10 000. The method does not adjust for major differences in dry weight which may alter the overall spore count estimates,

however if samples are collected at the same time in the morning when dew is still present on the pasture then, crudely, similar weights may be obtained

The multiple steps in the standard method provide three key stages which could influence the repeatability of spore counting. Firstly, there is within-farm and within-paddock variation, with differences in conditions across farms and paddocks influencing the development of *P. chartarum* (Keogh (1973). Secondly, 60 g of grass is selected from 200 g; variation within the 200 g could result in a difference in final spore count. Finally, only ~0.5 mL of wash water is selected from 600 mL of wash water.

There has been very little work to quantify the sources of variance in spore counts. The potentially large variation between samples from the same wash water is important as despite the findings of Di Menna and Bailey (1973) the recommended protocol is to do one spore count per grass sample. The aims of this study were to, firstly, quantify the variance in spore counts, arising from within the paddock, within the grass sample and within sample aliquots; and secondly to identify whether the within-paddock and within-sample variances could be attributed to the composition of those samples.

## 5.3 MATERIALS AND METHODS

This study was carried out on a convenience sample of four commercial dairy farms in the Waikato region of New Zealand. The four farms were well-established, easy-to-access dairy farms that milked approximately 150, 400, 450 and 500 cows on 62, 150, 165 and 125 ha respectively.

One easily accessible paddock was selected per farm . A total of 40 sampling sites within the boundaries of each study paddock were defined. At the start of the study a map of the paddock boundaries was provided by each participant herd manager and 40 equally spaced sampling points were sketched onto the map. The paddock was then paced out and 50 cm × 5 cm × 5 cm wooden pegs used to permanently identify each of the selected 40 sampling points, with the longitude and

latitude of each point recorded using a global positioning device (GPSMAP 64st, Garmin, Kansas, USA).

Each participant farm was visited by rural technicians once weekly for 19 weeks starting on 7 January 2013. Visits to each farm were scheduled to occur during the morning (typically between 10:00 and 12:00 a.m.). At each visit a large circular polythene pipe with a 1m radius was placed around the sample site identifier. A line was drawn from one side to the other of the circle and a sample of pasture of approximately 200 g (amount sufficient to fill 43.5cm x 18cm x 11cm paper bag) was cut at ground level with scissors along this line. Pasture samples were immediately placed into the disposable paper bag, labelled with farm, date and pegID and transported directly to the laboratory for processing. If there was limited pasture available then a smaller sample was taken from the sample site (~ 60g, sufficient to fill a 27cm x 13cm x7cm paper bag). If there was insufficient grass for even this sample, then the peg was marked as a no sample for that week. On the same date a traditional full paddock sample was taken walking from one corner to the other and taking 10 x 20 gram samples at even intervals along the diagonal.

On arrival at the laboratory the grass sample was manually mixed in the bag and 60 g of pasture selected and separated for composition analysis. A qualitative estimate of pasture dry matter was made by squeezing the sample and estimating the moisture content (wet, damp or dry). The pasture sample was then sorted into grass: green, yellow, and dead matter; clover: green, yellow, and dead matter; and weeds. Each of the seven sorted categories was then weighed. If there was insufficient organic material to register a weight, that component was recorded as <1 g.

***Spore counting***

The technique used was based on the standard method (Oldman and Di Menna, 1983), except that multiple measurements were made per pasture sample. Firstly, each 200 g pasture sample was separated into three separate 60 g samples which were then tested (one 60g sample was analysed for composition as explained above). Secondly, for each 60 g pasture sample, 10 aliquots of wash water were collected and counted. This meant that if a pasture sample of sufficient size was

available up to 30 spore counts could be made from the area around a single peg. Spores were counted using a microscope at 100× magnification. The total pasture spore count/g pasture was estimated by multiplying the number of observed spores by 10 000.

Traditional full paddock spore counts were determined using only one spore count test per 200 g sample of pasture.

All data were recorded on paper using a pre-prepared recording sheet. Data from these sheets were transferred onto an Excel spreadsheet on a weekly basis. .

***Statistical analysis***

Each spore count from each individual peg on an individual farm on an individual date was visualised by plotting the results as a dot plot. Using R for Windows (Version 3.1.2, https://www.r-project.org/) a non-parametric smoothed line of best fit and the uncertainty around the line of best fit was calculated and superimposed over the dot plot.

To visualise whether there were trends between pegs on different sampling dates a plot was made showing the spore counts on each sampling date, stratified by farm with a line connecting the individual pegs.

***Repeatability of spore count***

The repeatability of spore counting using multiple aliquots of the same wash water was assessed using intra-class correlation coefficient (calculated from a two-way mixed effects model with the individual grass sample as a fixed effect and sample order as a random effect; Shrout and Fleiss, 1979). This was within-subject standard deviation (calculated from a one-way ANOVA with individual grass sample as the independent variable and spore count as the dependent variable; Bland and Altman 1996). The homogeneity of this standard deviation was then tested by regressing the mean spore count for each individual grass sample against the standard deviation of the individual aliquots from that sample using both actual counts and log (y+1) transformed counts (Bland and Altman 1996).

***Agreement between spore counts from 1-3 samples and counts from 10 samples***

For this analysis, only data from individual grass samples with 6 or more tested aliquots of wash water were included; for the individual grass samples where <10 wash samples were taken the mean count per sample * 10 was used as the count for that grass sample ('total count').

The individual aliquot counts were then partnered with the total count of their grass sample (log transformed if >0) and the data separated by count of individual score (i.e. 0, 1, 2, 3 … up to ≥20). The log transformed total counts for each individual aliquot score category (from 1 to ≥20) were then tested to see if they were normally distributed (assessed using the Shapiro-Wilk test and q-q plotting). If this was the case, mean and standard deviation of the transformed data were calculated. These were then back-transformed to identify the 80 and 95% prediction intervals. For individual aliquots with 0-2 spores counted, where the data were not normally distributed, the 5, 20, 50, 80 and 95 percentiles were calculated from untransformed data, as were the data for aliquots with counts ≥20. For the latter as the number of aliquots was much smaller than for those with 0-2 spores, a 95% confidence interval for the 5 percentile was calculated using bootstrapping (2000 samples).

To create comparable models for the accuracy of prediction of one, two and three samples per wash, ten pairs and ten triplets were randomly selected from the results for each individual grass sample. For each dataset a generalised linear repeat measures model (with Poisson distribution and a log link) was then created with the total count as the dependent variable and the count from one, two or three samples as the independent variable. The difference between the predicted count for ten samples for each result (calculated from the model) and the actual count for the ten samples was then plotted against the mean of the predicted and the actual counts, with limits-of-agreement calculated using the method described by Bland and Altman (1999). This analysis was undertaken using SPSS Statistics 22

The accuracy of one grass sample per 200g sample as a predictor for the results of the average of three grass samples from the 200 grams was then assessed by calculating the difference between

the predicted count for three samples for each result and the actual count for the three samples. This was plotted against the mean of the predicted and the actual counts, with limits-of-agreement calculated using the method described by Bland and Altman (1999).

The accuracy of one traditional paddock sample in comparison to an average of all the peg spore counts on any given date was attempted by comparing the paddock spore count to the average of the peg samples if there were more than 15 peg results contributing to the average result. There were only 11 spore count results where 20 or more pegs were contributing to the average so the results were only compared descriptively.

The relationship between spore counts and individual grass components were assessed using a negative binomial mixed effects regression model. Descriptive results for individual grass component measurements were assessed initially before considering them for a multivariable model. Dead grass, yellow grass, dead clover, yellow clover and weed components were not included in the analysis as <5% of the data had values > 0 g.

Spore counts were log(y+1) transformed and the resulting counts were then compared to the measured grass components of green grass, clover, height and dry matter estimation and the final model was built using a backward selection of variables.

Linearity for pasture height was assessed by categorising it into three variables (<10cm, 10-14cm and >14cm) and plotting it against the log of the spore count. As it was deemed non-linear but significant, the categorical variable was used instead of the continuous height variable. Peg identification was added as a random effect to account for repeated measures of spore counts around pegs over time. For the final model the normality of residuals and the homoscedasticity of the dataset checked and confirmed to be acceptable.

Data analyses for the composition of pasture were conducted using the statistical package R version 3.1.2 (R Development Core Team 2014l R Foundation for Statistical Computing, Vienna, Austria). All other data analyses were conducted using SPSS 21.

## 5.4 RESULTS

Throughout the sampling period across all farms, spore counts, calculated from individual aliquots, ranged from 0 to 490,000 spores per gram of pasture (Table 5.1). Spore counts, overall, peaked at around 80-100 days after the start of the study (i.e. in April), but there was marked variation between farms both in spore count and in the change in spore count with time (Figure 5.1).

**Table 5.1: Descriptive statistics of estimated spore count concentrations for each of the five weeks of the sampling period.**

| Spore count category | *n* | Mean (SD) | Median (Q1, Q3) | Min, max |
|---|---|---|---|---|
| Week 1 | 799 | 6300 (14400) | 0 (0, 10000) | 0, 100000 |
| Week 2 | 1481 | 4700 (8200) | 0 (0, 10000) | 0, 60000 |
| Week 3 | 481 | 7300 (13300) | 0 (0, 10000) | 0, 100000 |
| Week 4 | 1470 | 6200 (16700) | 0 (0, 10000) | 0, 190000 |
| Week 5 | 1244 | 6200 (15300) | 0 (0, 10000) | 0, 120000 |
| Week 6 | 1250 | 9400 (17000) | 0 (0, 10000) | 0, 150000 |
| Week 7 | 280 | 3000 (6200) | 0 (0, 10000) | 0, 30000 |
| Week 8 | 360 | 1500 (4800) | 0 (0, 10000) | 0, 40000 |
| Week 9 | 460 | 7200 (15600) | 0 (0, 10000) | 0, 110000 |
| Week 10 | 240 | 5100 (8500) | 0 (0, 0) | 0, 50000 |
| Week 11 | 220 | 6200 (7600) | 0 (0, 0) | 0, 50000 |
| Week 12 | 350 | 7400 (10600) | 0 (0, 10000) | 0, 70000 |
| Week 13 | 410 | 40800 (91000) | 0 (0, 10000) | 0, 490000 |
| Week 14 | 730 | 39000 (66600) | 0 (0, 10000) | 0, 470000 |
| Week 15 | 440 | 4000 (42000) | 0 (0, 10000) | 0, 280000 |
| Week 16 | 1010 | 12500 (21400) | 0 (0, 10000) | 0, 180000 |
| Week 17 | 470 | 14500 (17300) | 0 (0, 10000) | 0, 140000 |
| Week 18 | 240 | 5100 (8200) | 0 (0, 10000) | 0, 40000 |
| Week 19 | 350 | 800 (3000) | 0 (0, 0) | 0, 20000 |
| Total | 12,784 | 11,300 | 0 (0, 10000) | 0, 490000 |

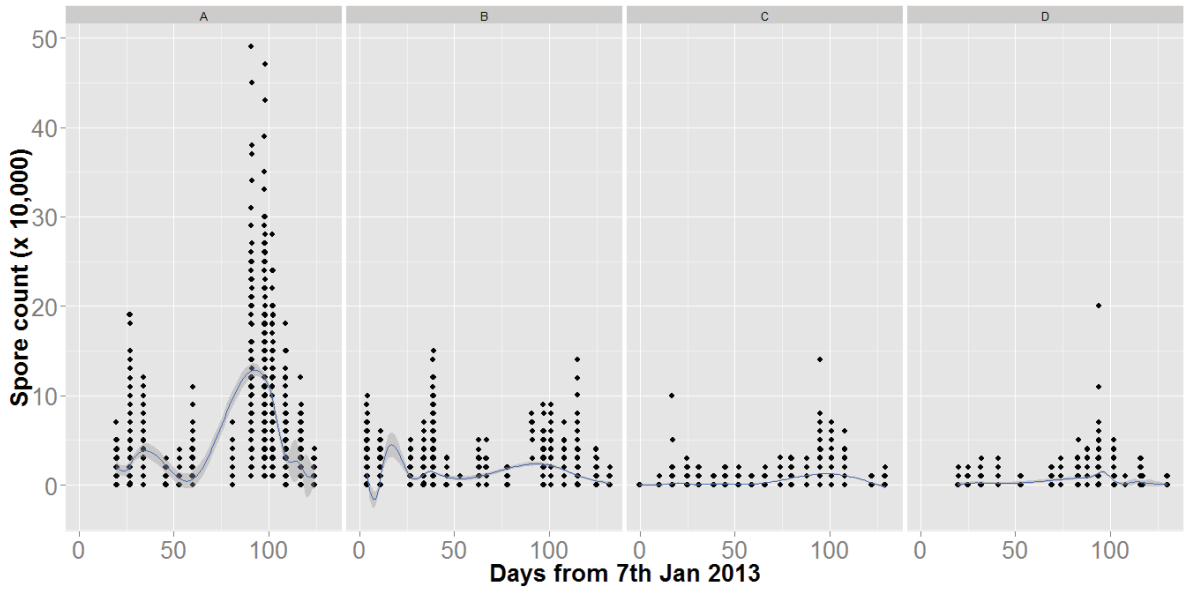


*Figure 5.1: Dot plot showing estimated spore count (× 10,000) per gram of pasture as a function of sampling date (expressed as the number of days from 7th January 2013), stratified by farm (labelled 'A' to 'D'). The blue line superimposed on each plot is a (non-parametric) smoothed line of best fit; the shaded areas indicate the uncertainty around the line of best fit.*

Within a paddock there was considerable variation in spore count, particularly on Farm A, which had the highest spore counts of all the farms. In addition, the variation in spore count with time was not consistent between pegs in the same paddock, with spore counts rising for some pegs while decreasing in others (Figure 5.2).

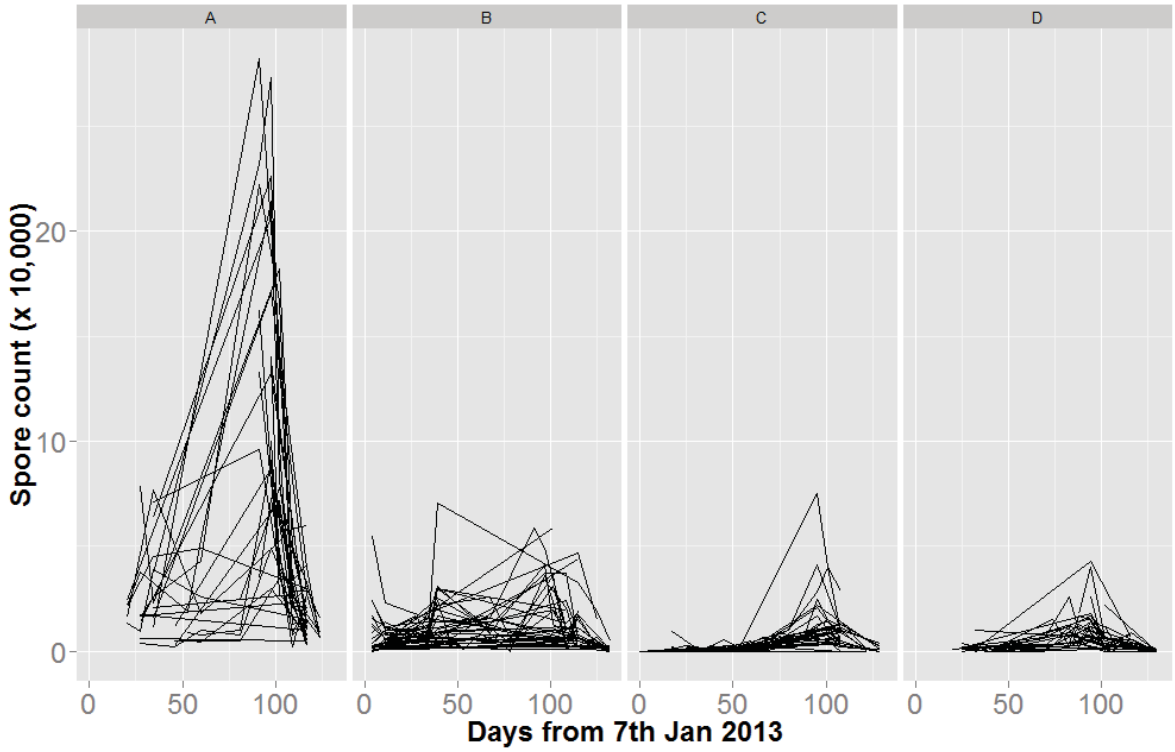


*Figure 5.2: Line plot showing the estimated spore count (x10,000) per gram of pasture as a function of sampling date (expressed as the number of days from 7th January 2013), stratified by farm (labelled 'A' to 'D').The lines connect samples from individual pegs on different sampling dates.*

***Repeatability of spore count***

When individual aliquot data were compared to the other individual aliquot data, ICC was 0.777 (95% CI 0.762 to 0.792). When individual aliquot data were compared to the mean of all the 10 samples, ICC was 0.972 (95%CI 0.97 to 0.974).

The within-subject standard deviation for the untransformed data was 1.836, but there was a significant association between the mean spore count for an individual sample and the standard deviation of the multiple aliquots ($r^2 = 0.65$ when data from all samples were included and 0.88 when data from samples with mean and standard deviation of 0 were excluded). Fig 3 shows the association; the line of best fit was standard deviation = 0.39* mean of aliquots +0.36.

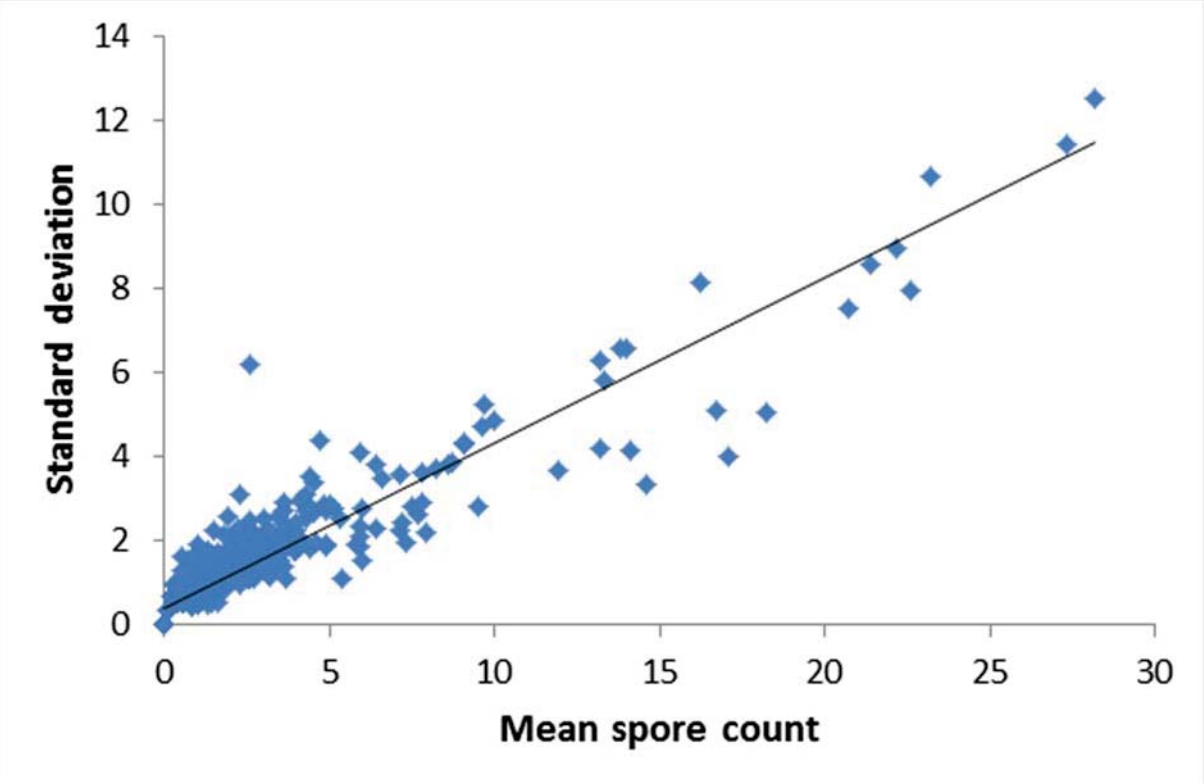


*Figure 5.3: Dot plot with line of best fit showing the association between mean spore count for multiple aliquots from one grass sample and the standard deviation of the counts of those aliquots.*

The within-subject standard deviation for the log (y+1) transformed data was 0.026, but although this transformation reduced the association between mean and standard deviation the association was still present ($r^2 =$ 0.3 when data from all samples were included and 0.15 when data from samples with mean and standard deviation of 0 were excluded). Thus no simple measurement of absolute repeatability (equivalent to that reported by Di Menna and Bailey (1973) ) could be calculated for this dataset.

The data for log total spore count were sufficiently close to normal distribution for individual counts of 3 – 19 spores. The back transformed mean and 80 and 95% prediction intervals are summarised in Table 5.2 for these individual aliquot spore counts, alongside the 5, 20, 50, 80 and 95 percentiles for individual counts of 0-2 and ≥20 spores.

**Table 5.2: Association between individual aliquot spore count and total count from 10 aliquots from the same grass sample**

| Individual count [a] | Geometric mean total count [b, c] | 95% PI [d] | 80% PI |
|---|---|---|---|
| 0 [e] | 1 | 0 - 11 | 0 -5 |
| 10 [e] | 8 | 1 - 26 | 3-14 |
| 20 [e] | 13 | 4 - 36 | 8 - 22 |
| 30 | 19.5 | 6.2 - 61.9 | 9.2 - 41.4 |
| 40 | 27.4 | 9.8 - 77.2 | 14.0 - 53.8 |
| 50 | 35.9 | 13.6 - 94.8 | 19.1 - 67.6 |
| 60 | 44.1 | 15.5 - 125.3 | 22.4 - 87.1 |
| 70 | 58.5 | 24.6 - 139.1 | 33.3 - 102.9 |
| 80 | 70.3 | 27.9 - 176.8 | 38.8 - 127.3 |
| 90 | 69.3 | 37.3 - 129.0 | 46.5 - 103.3 |
| 100 | 79.0 | 28.1 - 222.2 | 40.7 - 153.5 |
| 110 | 93.2 | 36.7 - 236.6 | 51.0 - 170.3 |
| 120 | 104.5 | 42.6 - 256.7 | 58.5 - 186.9 |
| 130 | 126.1 | 53.6 - 296.9 | 73.0 - 217.8 |
| 140 | 121.2 | 49.6 - 296.1 | 68.6 - 214.3 |
| 150 | 122.1 | 60.4 - 246.7 | 78.7 - 189.5 |
| 160 | 155.2 | 78.4 - 307.2 | 101.3 - 237.8 |
| 170 | 148.1 | 68.7 - 319.3 | 92.8 - 236.4 |
| 180 | 148.4 | 71.6 - 307.5 | 94.1 - 234.0 |
| 190 | 178.0 | 101.6 - 311.8 | 124.7 - 254.0 |
| ≥200 [e] | 214 | 132 [f] - 282 | 162 - 273 |

*[a], from one 10 mL aliquot; [b], from ten 10 mL aliquots; [c], to convert counts to estimated spore counts multiply total count (and its PI), and individual count by 1,000; [d], prediction interval; [e], data are medians and percentiles from untransformed data; [f], 95% confidence interval for this percentile was 47.3 to 138*

Figures 5.4-5.6 show the limits of agreement between the actual count of 10 samples and the counts from one, two and three samples. All plots show that the variability of results increases as the mean counts increase. The more counts used the closer the limits of agreement are between the two tests. Fig 5.7 shows the relative to the actual counts variation in predicted counts from the Poisson model for one, two or three samples based on the same actual count. This graph indicates that if the spore count based on the results of ten aliquots was 10,000 spores/g then 95% of results from only one water aliquot would be 2,000-50,000 spores where as if three water aliquots were counted, 95% of predicted results would be 5,000-20,000 spores/g.

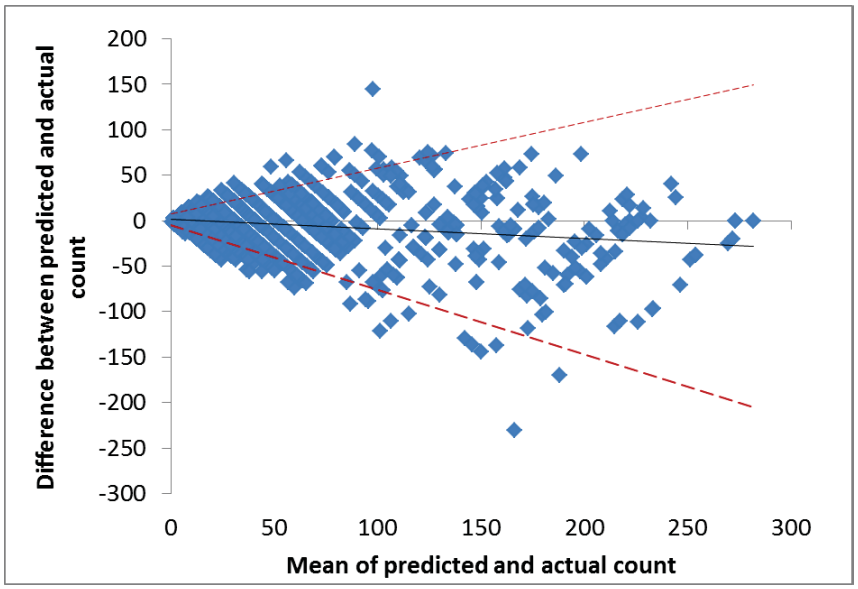


Figure 5.4: Bland and Altman limits of agreement plot for actual total count from ten aliquots per grass sample and predicted counts from one aliquot per grass sample. Solid line is line of best fit. Dashed line: 95% limits of agreement

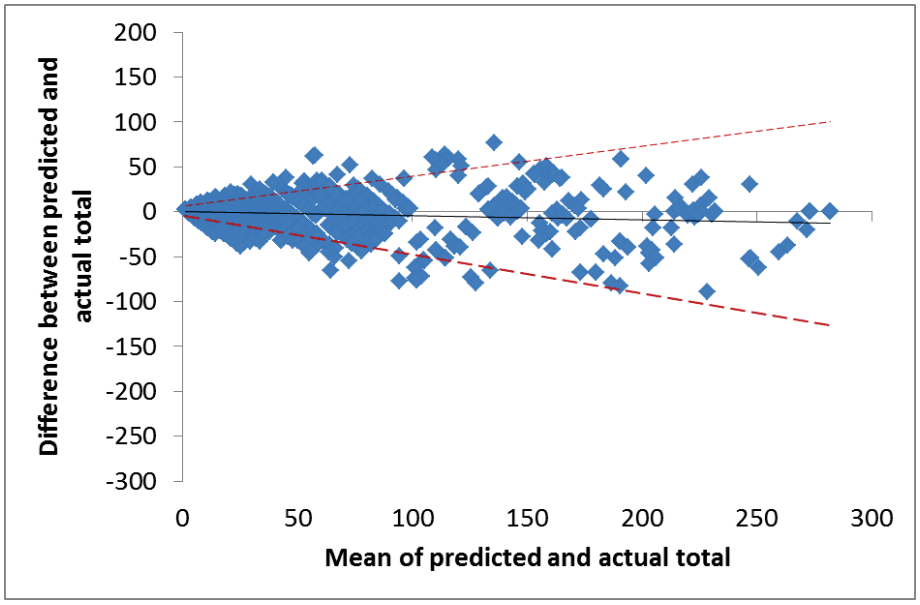


*Figure 5.5: Bland and Altman limits of agreement plot for actual total count from ten aliquots per grass sample and predicted counts from two aliquots per grass sample. Solid line is line of best fit. Dashed line: 95% limits of agreement*

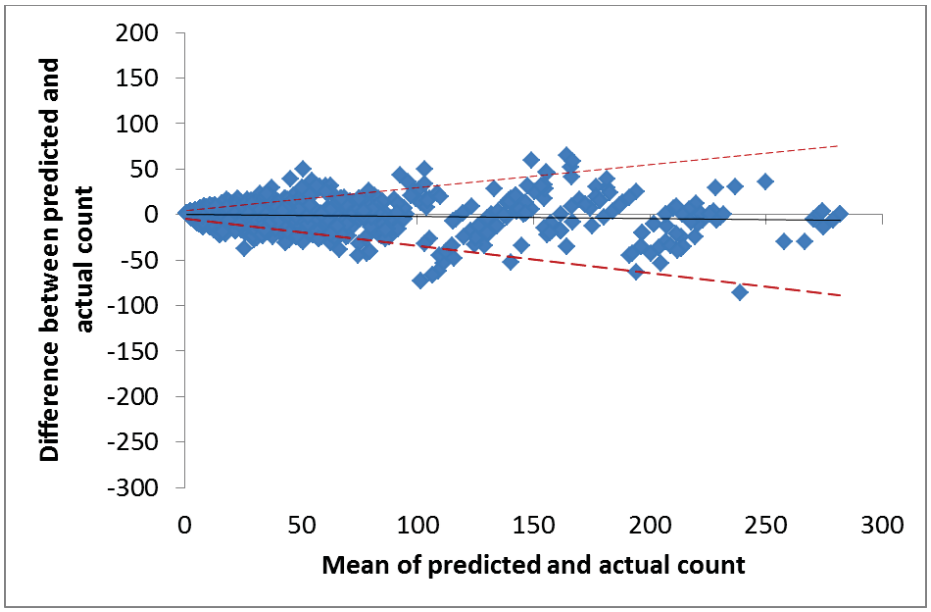


*Figure 5.6: Bland and Altman limits of agreement plot for actual total count from ten aliquots per grass sample and predicted counts from three aliquots per grass sample. Solid line is line of best fit. Dashed line: 95% limits of agreement.*

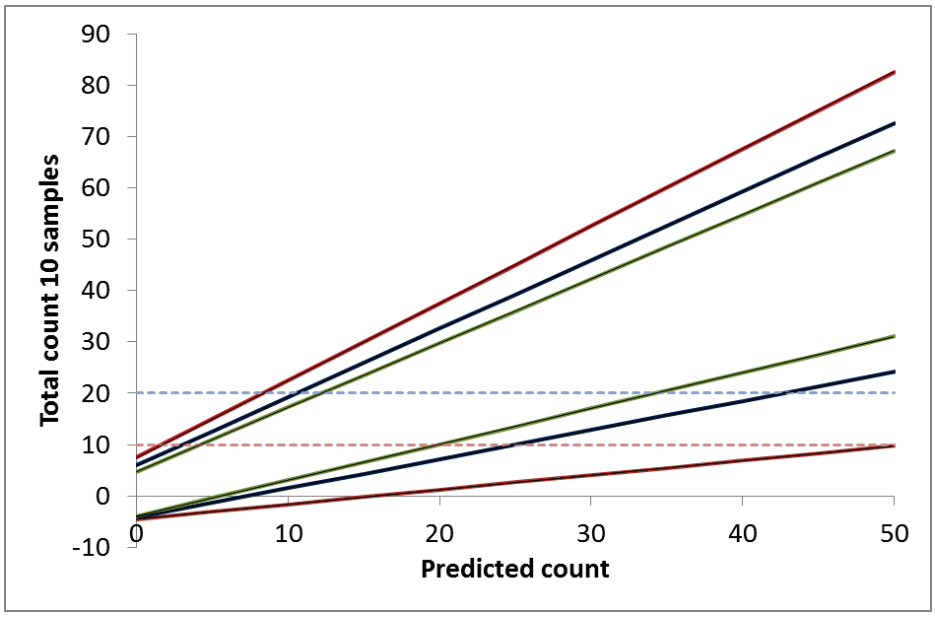


*Figure 5.7: Line plot showing the association between actual count from 10 aliquots per grass sample and predicted counts (from Poisson model) from one to three aliquots pre sample. This figure is based on the 95% limits of agreement plots. Red lines, upper and lower limits for one sample; blue lines, upper and lower limits for two samples; green lines upper and lower limits for three samples. Dashed lines mark total spore counts of 10 000 and 20 000; key levels of spore counts identified by DairyNZ 2014*

*To read the graph follow the line from the y-axis until it hits the line then read off perpendicularly the predicted count. The upper line marks the lower limit for the predicted count and the lower line the upper limit. Thus, 95% of predicted counts for a two-sample measure from 10 samples with a total*

*count of 20 will be between 11 and 44; while, 95% of predicted counts for a one-sample measure with a total count of 10 will be between 1.9 and 50.*

Table 5.3 shows, based on the limits of agreement analysis, the upper and lower limits of the expected total counts from ten samples which are associated with predicted counts of 10, 20, 30, 40 and 50 spores (equivalent to spore counts of 10 000 to 50 000).

**Table 5.3: 95% limits of agreement for mean counts from one to three samples for total counts from 10 samples from the same wash water from an individual grass sample.**

| | Predicted count | | |
|---|---|---|---|
| Total count | One sample | Two samples | Three samples |
| 10 | 0-21 | 2-19 | 3-16 |
| 20 | 2-35 | 7-32 | 10-29 |
| 30 | 5-49 | 12-45 | 17-42 |
| 40 | 7-63 | 18-47 | 26-54 |
| 50 | 10-76 | 23-70 | 30-66 |

*Interpretation: For a grass sample where the total spore count from 10 samples was 20, the predicted spore counts (based on the Poisson model) will 95% of the time, be between 2 and 35, if one aliquot is measured and between 10 and 29 if three aliquots are measured.*

The limits of agreement plot for the comparison between the counts from one grass sample and the total count from three grass samples from the same site is shown in Figure 5.8. As was found when comparing the results from 10 aliquots to those from one, two and three; there was a marked increase in variance as the mean spore count increased, which meant that the limits of agreement increased markedly as mean spore count increased. Using only data from samples with a mean spore count of ≤100 spores did not markedly decrease the effect of mean count on the limits of agreement (i.e. red line on Figure 5.8 is only moderately different from dashed line).

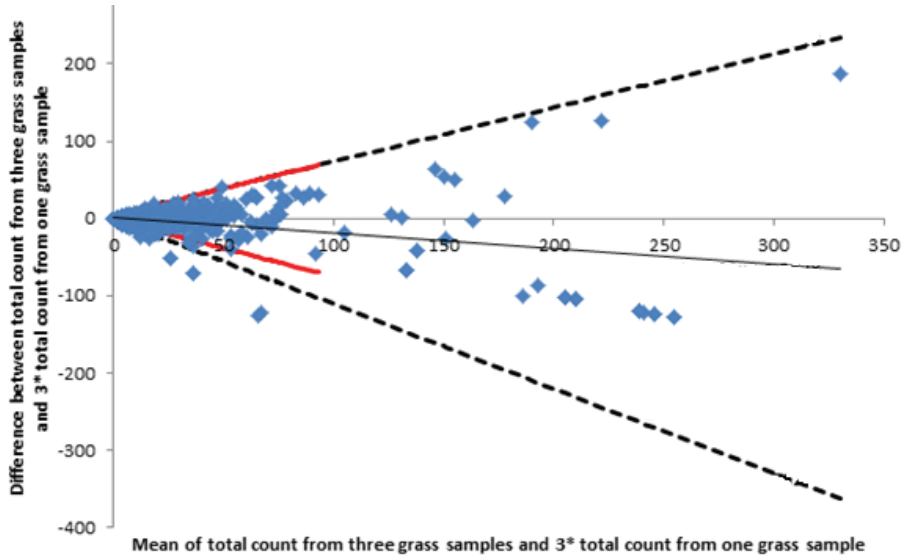


*Figure 5.8: Bland and Altman limits of agreement plot showing agreement between total spore count from three grass samples and 3* spore count from one of those three grass samples. Solid line is line of best fit; dashed lines are 95% limits of agreement. Red lines are 95% limits of agreement calculated using data from grass sample where mean of the two counts was ≤100.*

*Note: to convert these data to estimated spore count / g of pasture multiply by 1000/3*

Table 5.4 compares, for expected spore counts of 10,000, 20,000 and 50,000 spores / g of pasture the spread of the 95% limits of agreement for one sample vs three grass samples and for one and three vs. 10 aliquots from the same grass sample. Over the range from 10,000 to 50,000, spores per g of pasture, the width of the limits of agreement for one vs three grass samples at a site were similar to or higher those for one vs 10 aliquots.

**Table 5.4: Comparison between limits of agreement from testing one versus three grass samples from the same site and testing one or three aliquots vs 10 aliquots from one grass sample**

| Total Count (spores/g of pasture) | Spread of LOA for 1 vs 3 grass samples (fig below)* | Spread of LOA for 1 vs 10 aliquots§ | Spread of LOA for 3 vs 10 aliquots† |
|---|---|---|---|
| 10 000 | 20000 | 26000 | 13000 |
| 20 000 | 31000 | 32000 | 19000 |
| 50 000 | 91000 | 8100 | 36000 |

*, from Figure 5.8; §, from Table 5.2; †, from table 5.3.

The descriptive analysis comparing the average of more than 15 peg samples in a paddock in and the traditional paddock sample indicates that there was little variability between these two methods of grass sampling (Table 5.5).

**Table 5.5. Descriptive results from a comparison between the average spore counts from multiple peg sites and a traditional paddock spore sample.**

| Paddock | Number of pegs | Mean individual peg count | Paddock spore count |
|---|---|---|---|
| 1 | 40 | 5808 | 0 |
| 2 | 21 | 1143 | 0 |
| 3 | 33 | 522 | 0 |
| 4 | 22 | 636 | 0 |
| 5 | 28 | 5214 | 0 |
| 6 | 16 | 3188 | 0 |
| 7 | 35 | 18981 | 10000 |
| 8 | 29 | 12024 | 10000 |
| 9 | 23 | 638 | 10000 |
| 10 | 21 | 8160 | 20000 |
| 11 | 33 | 7727 | 20000 |
| 12 | 21 | 14814 | 20000 |
| 13 | 22 | 31682 | 40000 |
| 14 | 27 | 15927 | 50000 |

The multivariable analysis (Table 5.6) showed that in comparison to pasture heights of less than 10cm increased height of pasture was associated with a lower spore count (10-14cm, -0.72 spores/g; >14cm, -0.88 spores/g). It also showed that in comparison to wet pasture, medium dry matter pasture was associated with a higher spore count than wet grass (5.1 spores/g; p=0.005).

**Table 5.6. Multivariable linear regression model of the grass components affecting the geometric mean spore counts (spores/gram pasture)**

| Coefficients | Estimate | 95%CI | P |
|---|---|---|---|
| Intercept | -0.97 | -0.99,-0.91 | <0.001 |
| | | | |
| Height <10cm | | | |
| Height 10-14cm | -0.72[a] | -0.85,-0.44 | <0.001 |
| Height >14cm | -0.88 | -0.96, -0.67 | <0.001 |
| | | | |
| Grass Dry Matter (Wet) | *Reference* | - | |
| Grass Dry Matter (Medium) | 5.1[b] | 0.71,20.4 | 0.005 |
| Grass Dry Matter (Dry) | 2.6 | -0.50,24.9 | 0.20 |

a. Interpretation: In comparison to pasture with less than 10cm in height, pasture between 10-14cm had 7,200 spores/gram pasture.

b. Interpretation: In comparison to wet pasture, medium dry matter pasture had 51,000 more spores/gram of pasture.

## 5.5 DISCUSSION

This study has critically looked at the spore counting method that is currently recommended in New Zealand. In particular it has looked at the key reasons why estimated spore counts vary, and evaluated whether pasture factors have a major impact on this variability.

The initial intention of this study was to find a simple single measure of variability that could be used alongside every spore count to provide farmers and advisors an estimate of how much confidence to have in a single spore count. However this was not possible, as the repeatability of spore counting decreased dramatically as spore counts increased whether the repetition was from multiple aliquots from the same sample or multiple grass samples from the same site.

As expected there were major variations between farms in spore count and in the variability of spore count. This is consistent with many previous reports (Parle and Menna 1978) and confirms the importance of individual farm testing if spore counting is to be used for monitoring the risk of FE and for the timing of implementation of control programmes

In addition to the between-farm difference, there was also a large variation in spore counts between samples taken at different sites (pegs) within a paddock. This variation in spore count across the paddock is likely to be due to differences in microclimate across the paddock affecting the growth of *P.chartarum* (Brook 1963). In this study, differences of up to 500,000 spores/g were seen between

pegs in the same paddock. This was higher than the variability reported by Di Menna and Bailey (1973) who reported that spore counts ranged from 15000-90000 spores/g when separate samples were taken from five points in a paddock with long uneven pasture. The larger difference may simply be a reflection of chance with this study sampling more paddocks on more occasions with more samples per paddock and also having paddocks with much higher spore counts than the paddock evaluated by Di Menna and Bailey (1973).

This finding strongly supports the current recommendation of taking multiple grass sample collections across a paddock rather than just sampling one site. The current recommendation is to take these multiple samples along a diagonal; there were too few occasions when there were ≥15 pegs which had sufficient grass for sampling to compare this procedure statistically with sampling at multiple sites around the paddock. However, there were clear differences between the recommended technique and the mean results from multiple pegs. This was particularly so for the six paddocks where the standard method identified 0 spores; in none of those paddocks was mean peg spore count = 0. For paddocks where spores were identified using the standard method, the spore count using the standard method was higher than mean peg spore count for all but one of the eight paddocks. This dataset is not large enough to establish how well sampling on a diagonal reflects spore count from sample points spread across the paddock, but the low number of paddocks in this study with ≥15 samples per paddock shows how difficult a true random sample would be to achieve. Sampling on a diagonal may not be ideal but true random sampling is not feasible because it is a more prescribed procedure. Random sampling reduces the chance of choosing non-representative sampling sites, which may occur if 10 or more sites are selected ‘throughout’ the paddock.

The large variation across a paddock means that intake of spores can vary significantly between cows. Based on the differences seen in this study, the location where a cow grazes could significantly affect its risk of getting FE. This difference between spore counts and spore intakes has led to the development of faecal spore counting as a means of monitoring FE risk. However we agree with

(Anonymous 2013) that currently there is insufficient information to guide the interpretation of faecal spore counts; furthermore, there are no data on the reliability of faecal spore counting.

This study has identified that there is significant variation within the wash water from an individual pasture sample. This variation can be looked at in two ways; firstly, the accuracy of an individual aliquot result as a predictor of the concentration of spores in the wash water. The prediction interval analysis (Table 5.2) suggests that when the pasture spore count estimated from an individual aliquot is 10,000 spores/g, 95% of the time the estimate from 10 aliquots will be between 1 and 26,000 spores/ g pasture. When the estimate from an individual count is 20,000 spore/g, 95% of the time the estimate from 10 aliquots will be between 4 and 36,000 spores/g pasture (Table 5.2). At these spore counts, the results from an individual aliquot agree moderately well with the results from 10 aliquots. However, as spore counts increase, the prediction intervals increase markedly between spore counts from one and ten aliquots also increase.

Table 5.3 and figure 5.7 illustrate the effect of increasing the number of aliquots tested on the agreement with total counts from ten aliquots. This analysis used a repeat measures model with Poisson distribution and a log link to allow direct comparison of the three sampling strategies, so the results in Table 5.3 are not directly comparable with those in Table 5.2. In particular, although the limits of the agreement for one aliquot (Table 5.3) are smaller than the prediction intervals (Table 5.2), the model is more likely to underdiagnose true spore count.

The second way of looking at the spore count variability is looking at the range of possible individual aliquots when the total count from 10 aliquots is a particular value. This analysis is shown in Figure 5.7. Using this figure it can be seen that if the estimated spore count from the total count from 10 aliquots is 10,000 spores/g, then 95% of the estimates from individual aliquot spore counts will be between 0 and 50,000 spores/g of pasture. For an estimated spore count from 10 aliquots of 20,000 spores/g, the equivalent figures will be 10,000- ~100,000. Both of these analyses show clearly that the agreement between spore count estimates from one aliquot per grass sample and 10 aliquots per grass sample is poor.

Nevertheless, poor agreement may still be sufficient agreement for use on-farm. As can be seen in Table 5.2 the key issue in terms of agreement is that individual aliquot counts tend to overestimate total count from 10 samples; the data suggests that almost 10% of individual aliquots which have five or more spores will come from a pasture with a true spore count <20, 000. In contrast, underdiagnosis of true spore count was unlikely particularly at low counts (<20 000/g); for example only ~5% of individual aliquots where only one spore is identified will come from a pasture with a true spore count >20, 000.

It might be argued that the over diagnosis of spore counts is not a concern when spore counts are being used for monitoring purposes, particularly in regard to identifying when to begin FE prevention programmes. For example, if the data from one aliquot suggests that there is a spore count of 50,000/g when the true spore count is actually only 10,000 spores/g, then the main effect will be that farmers will start their FE management program earlier than necessary. While this is not as much of a welfare concern as starting too late, this can still be a problem when farmers are using slow-release boluses. These have a limited lifespan, so if they are used too early it is unlikely that they will provide protection for the whole FE risk period. However, they are expensive and farmers may be reluctant to retreat animals later in the season.

For the threshold of 50,000 spores/g of pasture the lack of accuracy is more of a problem. Individual aliquots with spore count of 30,000 spores/g have a ~5% chance of coming from a pasture with 50,000 spores/g (for 40,000 spores/g the equivalent figure is >10%). This under estimation could have significant welfare implications. We recommend that when using spore counting to stop FE prevention programmes, farmers should have consistently low results for at least three weeks before assuming that spore counts are below dangerous levels.

Increasing the number of aliquots from one to two or from two to three, markedly reduces the limits of agreement, reducing both over and under diagnosis of significant thresholds. The data from this study thus supports the conclusion that the current recommendation of counting the spores in a

single aliquot of ~0.5 mL taken from 600 mL of wash water used to wash 60 g of pasture does not result in a sufficiently accurate spore count.

The only previous measurement of repeatability of spore counting was by Di Menna and Bailey (1973) who counted the spores in ~2 mL taken from 250 mL of wash water used to wash 25 g of pasture. As they did not report the range of spore counts in their test samples, direct comparison with the results of the current study are not possible. However, the mean differences they reported between samples (9,000 from two aliquots from the same wash water and 8,000 for two separate washes made from the same pasture) were similar to the differences seen in this study between 10 counts and one count when total spore count was between 0 and 20,000. This suggests that the between- sample variability reported by DiMenna and Bailey (1973) was less than seen in this study; this may have been a result of the greater volume of wash water which was spore counted (2 mL in DiMenna and Bailey (1973) vs. 0.5 mL in the current study).

Changing from measuring one to three aliquots is a simple and easy change which will require little extra time in the laboratory so we recommend that this should become the standard procedure for farm monitoring purposes. Further research is required to establish the optimal volume to be tested.

Table 5.4 shows that the spread of the limits of agreement for 30 aliquots (10 per sample from 3 grass samples collected from a single one metre radius area) compared to 10 aliquots (from one grass sample) were similar to those from one vs 10 aliquots from the same grass sample. This suggests that variation between spore count results from a single 60 g pasture sample in comparison to the average spore count result from three separate 60 g pasture samples was as high as between one and 10 aliquots of the same wash water. This is even though the three samples were taken from the same area.

Changing the number of grass samples tested significantly increases the time required as preparation of wash water is a much longer process than selecting an additional aliquot of wash water. There is likely to be reluctance to testing multiple grass samples per site, particularly in addition to counting multiple aliquots per wash water. Attention needs to be paid to identifying

methods of improving the mixing of samples to ensure that individual samples are more representative of the whole, both before selection of 60 g of pasture from the 200 g and of the wash water.

One of the key aims of this study to assess whether differences in pasture components were responsible for the high variability in spore counts within a paddock. However, the quantity of many of the different pasture components was too small to be analysed, and the only significant relationships identified were between decreasing spore counts with increasing height of pasture and increasing spore counts when medium dry matter pasture was compared to wet pasture. The late summer and autumn of 2013 was very dry with very little pasture growth over the months of the study. This meant that on most sampling occasions, most pegs had too little pasture for a sample to be collected. When there was sufficient pasture for sample collection it was often new growth which had had little time for the development of dead and yellow matter. This was likely to contribute to the relationship seen between height and spore count.

Weather was also likely to be the cause of the negative association between pasture height and spore count. In this season, in taller pasture, more of the 60g sample would have been green matter, which has a lower spore load, than base (Smith and Crawley 1962) as the increased height was due to new growth, whereas in better growing seasons, height can be associated with older pasture that has not been grazed before death and decay occurred at the base.

Higher spore counts were found on pasture that had a medium dry matter estimation in comparison to wet pasture. This was likely due to the fact that the wet pasture had recently been rained on. This may have altered the weight of pasture so comparatively less pasture was included in a 60 gram sample than dry pasture. It is also possible that the rain detached spores to other areas or to lower down at the base of the pasture. Smith and Crawley (1964) showed that the impact of large water (or rain) drops could be a potent means of detaching spores of P. chartarum from plant material (litter) on which they were produced. The water films on the herbage leaves can aid in moving spores up or down leaves for short distances.

However Smith and Crawley (1964) also showed that the air-shock wave and turbulence following water drop impact would be adequate for local spread of the spores, i.e. to surrounding green leafage in pastures in the absence of surface wind currents. This is supported by other studies looking at spores dispersion of fungi suggesting that large transient increases in the concentration of some dry airborne spores coincident with the start of rain suggested that the first raindrops to wet surfaces might disperse spores other than in splash droplets or by wetting fructifications (Hirst and Stedman 1963).

It is important to note, that despite the small quantities of dead and dying matter there was still considerable variation in spore counts which indicates that unmeasured variables such as soil temperature, humidity, location and topography of individual peg sites were also likely contributors to the variation in addition to grass components.

## 5.6 CONCLUSION

This study has identified that the repeatability of spore counting is poor, either when comparing different 60 g grass samples selected from a combined 200 g grass sample or comparing between multiple aliquots selected from the same wash water. If spore counts are to be used for monitoring purposes to identify when to start and finish FE prevention programmes we recommend that at least three aliquots per wash water are selected and that for stopping programmes at least 3 low spore counts must be recorded.

The variability across and between paddocks combined with the inaccuracies associated with sampling the collected grass and sampling the wash water means that, especially when FE risk is moderate to high, the current standard method of spore counting does not provide a reliable method of identifying the likely intake of sporidesmin by dairy cattle. Alternative methods of measuring actual sporidesmin intake need to be developed; faecal spore counting is currently the only feasible alternative but it needs further validation and repeatability testing to identify the best protocol. The development of better methods of identifying likely sporidesmin intake would be of

significant benefit in research which uses natural intoxication, as this study has shown that the standard method of spore counting provides extremely inaccurate estimates of sporidesmin intake in such circumstances, but is also likely to be of benefit for on-farm monitoring of FE risk.

# The effectiveness of current facial eczema management protocols used on dairy farms in New Zealand

## 6.1 ABSTRACT

**Aim:**

To document current practices used to manage and prevent facial eczema (FE) on 107 North Island dairy herds, and determine the effectiveness of each practice.

**Method:**

Nine veterinary clinics located in Bay of Islands (BOI), Whangarei, North Waikato, Waikato, South Waikato, Taranaki, Bay of Plenty and Manawatu randomly selected 10 or 20 farms (depending on the practice) to participate in the study once the regional spore counts started rising towards 30,000 spores/g pasture. Herd managers selected 10 cattle that were representative of the herd within 1-5 days of being contacted. The cattle were weighed and blood sampled by the veterinarian. The blood samples were sent to New Zealand Veterinary Pathology (Hamilton, New Zealand) for estimation of serum zinc concentration and GGT activity. A survey on farm management practices relating to prevention of FE was completed with the herd manager by the veterinarian. Pasture samples were collected from four “representative” paddocks from each farm and submitted for spore count estimation.

**Results**

Of the 1071 cows tested, 79 cows (7.3%; 95%CI 5.8-9.0) had evidence of moderate to severe liver damage (GGT>300IU/L), while 35/107 farms (33%;95%CI 24.2-42.8) had one cow or more out of 10 with GGT activity > 300 IU/L. Of the 911 cows that were being treated with zinc, only 288 (32%; 95% CI 28.6 – 34.7) had serum zinc concentrations within the protective range (20-35 µmol/L). A total of 32/911 (3.5%; 95%CI 2.4-4.1) had serum zinc concentrations >35 µmol/, while 623/911 (68.3%; 95%CI 65.2-71.3) had values <20 µmol/L and therefore not protective against FE. Possible reasons for such a high prevalence of sub-clinical FE and poor zinc concentrations were 1) timing of management programmes; 2) inadequate dose rates; 3) inadequate knowledge of management methods; 4) inadequate understanding of the disease; and 5) lack of a consistent message from veterinarians and rural professionals on the management of FE.

**Conclusion**

This study has highlighted that FE management on dairy farms in New Zealand could be substantially improved, principally through farmers getting more information on the success of their FE management programmes and responding when tests show that FE management is not effective.

## 6.2 INTRODUCTION

Facial eczema (FE) is a common and well documented problem faced by dairy farmers throughout the North Island and the top of the South Island of New Zealand (Andrew 1957). It is caused by ingestion of spores containing the toxin sporidesmin, which is produced by the saprophytic fungus (*Pithomyces chartarum)* which grows on dead and decaying pastures when weather conditions are warm and humid (Brook 1963). Sporidesmin ingestion leads to liver and bile duct injury (Mortimer 1963; Mortimer and Stanbridge 1968). This liver damage means that phylloerythrin, a photodynamic breakdown product of chlorophyll, is no longer effectively excreted but circulates in the blood stream, and can cause photosensitivity and lesions in unpigmented skin (Clare 1944).

Losses from FE arise from deaths, condemnation of carcasses and impaired productivity including loss of live weight, reproductive failure and decreased milk production. In dairy cattle, Towers and Smith (1978) demonstrated a 25% drop in daily milk volume when cows were artificially dosed with sporidesmin, while Smith and Embling (1983) showed a reduction of 10% in milk volume when cows were naturally exposed to long periods of low spore counts (0-90,000 spores/g) on pasture. The economic impact of FE has been estimated at $30 million annually with zinc prophylactic treatment and if not used the cost increases to $97 million (Dennis and Amer 2014).

Several methods have been developed to control facial eczema. These comprise understanding the farm risk through recognition of toxic pastures and the use of alternative "safe" pastures, use of pasture fungicides and the administration of high doses of zinc or zinc salts either through the water, in the feed, as an oral drench or in slow release capsules.

Currently, pasture spore counting is the method which is most commonly used to identify the level of risk of FE; with a 60 g pasture sample being collected from multiple sites along a line in a paddock, mixed with 600 ml of water and the number of spores in a small aliquot of the wash water being counted (Oldman and Di Menna 1983a). As this wash water gives the spore count per gram of herbage it was initially anticipated that this method would account for animal intake and thus be a useful indicator for district warnings (Di Menna and Bailey 1973). However, the FE risk faced by susceptible livestock does not just depend on the pasture count but also the type of stock at risk, the stage of lactation or growth, grazing management, and length of time spore counts remain high (Marbrook and Matthews). These factors need to be taken into account when assessing FE risk. Furthermore, significant variability within and between paddocks, as well as between farms means that a single district level of spore count information is not useful for FE control (Marbrook and Matthews). Spore counts should therefore, be used primarily to determine the rising or falling trend on a specific farm of interest, with district information being used to trigger the start of individual farm monitoring.

Fungicides have been used to control FE by killing *Pithomyces chartarum* on the pasture. The first fungicide which was shown to suppress the growth of *Pithomyces chartarum* was thiabendazole, a

substituted benzimidazole anthelmintic (Robinson *et al.* 1964). Sinclair and Howe (1968) showed that multiple sprays with thiabendazole were more effective than a single spray. Currently, carbendazim, another benzimidazole fungicide, is the active ingredient in the fungicides sold commercially for FE control, based on the results of (Wallace 1976) who showed that carbendazim gave greater control of spore counts for longer periods when used before and during peak spore counts compared to two other benzimidazoles, thiophanate and benomyl. Fungicides are most effective at controlling spore counts when pasture treatment begins prior to the rise in spore numbers (Wallace 1976).

The most commonly used method of FE control is in high intakes of zinc (15-20mg/kg live weight) (Towers and Smith 1978; Munday *et al.* 2001). This method of FE control was first discovered in the early 1970s by Gladys Reid of Te Aroha. Her discovery was confirmed by Towers (1977b) who found that zinc supplementation protected ruminants from FE when they were fed approximately 25 times their daily requirement. The protective effect of zinc has been found to be related to its ability to inhibit the generation of a superoxide radical by sporidesmin (Munday 1984). Effective control of facial eczema using zinc requires regular zinc supplementation (usually daily) before pasture becomes toxic (Smith and Embling 1999). Various routes of zinc administration have been used. The most commonly used methods are supplementation via drinking water or feed or administration via an oral drench or slow release capsule.

Whichever method of FE control is used it is important to be able to assess the effectiveness of the management programme. The most commonly used method of determining the effectiveness of FE control is by measuring serum gamma glutamyl transferase (GGT) activity. Serum GGT activities are indicative of cholestasis and bile duct damage and have been shown to rise after sporidesmin dosing of sheep and cattle (Ford 1974) and to be related to liver damage scores on post-mortem examination (Towers and Stratton 1978). GGT activity has been used to assess the severity of sporidesmin intoxication for over 45 years (Marbrook and Matthews).

When zinc supplementation is used to control FE, monitoring zinc intake (usually via serum zinc concentrations) should be used in addition to measurement of GGT activity. As zinc-based FE control requires pharmacological levels of zinc to be supplemented, the standard thresholds used for diagnosing zinc deficiency are not suitable for determining that zinc intake is sufficiently high to protect against FE. The currently used target serum concentrations are based on the findings of Smith (1987), who reported that serum zinc concentrations between the ranges 20-35µmol/L were sufficient for facial eczema protection..

However, despite the widespread use of FE control, particularly zinc supplementation on dairy farms and the availability of proven monitoring methods, outbreaks of FE continue to occur in dairy herds throughout the affected regions causing a significant production and welfare impact. This suggests that on too many dairy farms FE management protocols are not being applied correctly or, if they are being correctly applied, drug delivery mechanisms are faulty. The aims of this study were to document current practices used to manage and prevent FE on North Island dairy herds, and determine the effectiveness of each practice.

## 6.3 MATERIALS AND METHOD

The study was approved by the AgResearch Animal Ethics Committee (Ruakura), application 13024. A total of nine veterinary clinics were selected on the basis they undertook weekly regional pasture spore counts and were interested in being involved in the study. The clinics were located in Bay of Islands (BOI), Whangarei, North Waikato, Waikato, South Waikato, Taranaki, Bay of Plenty and Manawatu.

When weekly regional spore counts were trending upwards and approaching 30,000 spores/g of pasture in a given practice area, participating veterinarians randomly selected 10 or 20 farms (depending on the practice) from their practice client list. Herd managers from these farms were contacted and invited to participate in the survey within 1-5 days of being contacted.

Herd managers selected 10 normal cows from their herd; five that were ≤ 4 years of age and five that were > 5 years. Selected cattle were drafted from the main milking herd and held for examination at approximately 10 am. Blood samples were taken from the tail veins of the 10 cows into tubes with no anticoagulant. These were sent, within 12 hours of sampling, to New Zealand Veterinary Pathology (Hamilton, New Zealand) for estimation of serum zinc concentration (Anonymous 2013) and GGT activity. GGT was calculated by an enzymatic colorimetric assay measuring the amount of 5-amino-2-nitrobenzoate that results when GGT transfers the y-glutamyl group of L-y-glytamyl-3-carboxy-4-nitroanilide to glycylglycine. This was measured photometrically using a Roche automated clinical chemistry analyser (Thomas 1992). The ten cows were then weighed on electronic weigh scales. The survey on farm management practices relating to prevention of FE was then undertaken with the herd manager by the veterinarian (Supplementary material).

Pasture samples were collected from four paddocks on each farm and submitted for spore count estimation. The four paddocks chosen were "representative" of the whole farm by varying the location and contour. Pasture samples were taken by walking in a diagonal line from one corner of a paddock to the opposite corner. Approximately 20 g of pasture was collected by cutting pasture at the base at 10 locations along the diagonal line.

The collected pasture/forage (~200 g) was then gently mixed inside the collection bag, and 60 g removed. This was then added to 600 mL of water in a plastic container. The container was shaken vigorously for 3 minutes before the pasture sample was removed. A sample of the remaining wash water was then collected using an eye dropper while gently agitating the container back and forth. A cover slip was applied over the grids of a hemocytometer slide and both sides were filled with the wash water. Spores were counted using a microscope at 100x magnification. The total pasture spore count/g pasture for each paddock was estimated by multiplying the number of observed spores by 10,000.

***Statistical analysis***

A herd was classified as having evidence of facial eczema damage if one or more sampled cows had a GGT serum activity > 300 IU/L (indicative of moderate to severe liver damage caused by FE; (Towers and Stratton 1978) .

A herd was classified as having inadequate zinc intake to provide effective FE protection if three or more cows out of the 10 sampled had serum zinc concentrations <20 µmol/L. Confidence intervals for survey questions were calculated using the EpiR package in the statistical software R v3.1.2 (R Foundation for Statistical Computing, Vienna, Austria).

All responses (factors) to the survey questions that had a plausible relationship to the outcome of adequate zinc intake were compared using a multivariable GLM logistic regression in the same statistical software. The multivariable model was built using a reverse stepwise approach. Confounding and interaction was assessed throughout the model building process. The survey questions were not compared to the outcome of liver damage because the major influence of this outcome is spore counts at least two weeks or more prior to the sampling (which we did not collect). The author felt that this analysis would not truly represent the risk factors without including this major confounding variable.

## 6.4 RESULTS

A total of 1071 Jersey, Friesian, Holstein Friesian, Ayrshire or crossbred cows were sampled from 107 farms. Cows were lactating, ranged from 3-11 years of age, had live weights between 299 kg to 776 kg and were predominantly grazing ryegrass-based pastures. Other foods being fed included maize silage, palm kernel expeller, chicory, plantain, meal and molasses.

***Pasture Spore Counts***

The majority of the 417 spore counts made for this study were between 0 - 20,000 spores/g pasture. However, spore counts varied between regions, between farms within regions and between paddocks within a farm (Figure 6.1).

Of the 107 herd managers, 103 (98%; 95%CI 93.3-97.8) stated that they had access to regional spore count data. A total of 79/107 herd managers (75%; 95% CI 66 - 83%) reported that they retrieved regional spore count information from their local veterinary clinic. Others retrieved spore count information from local newspapers, RD1/Farmlands and/or farm advisors.

Only 35/107 herd managers (33%; 95% CI 24 - 43%) reported that they measured spore counts on their own farm; of which only five (4.7% of all surveyed farmers; 95%CI 0.7-8.7) monitored the same paddocks each time.

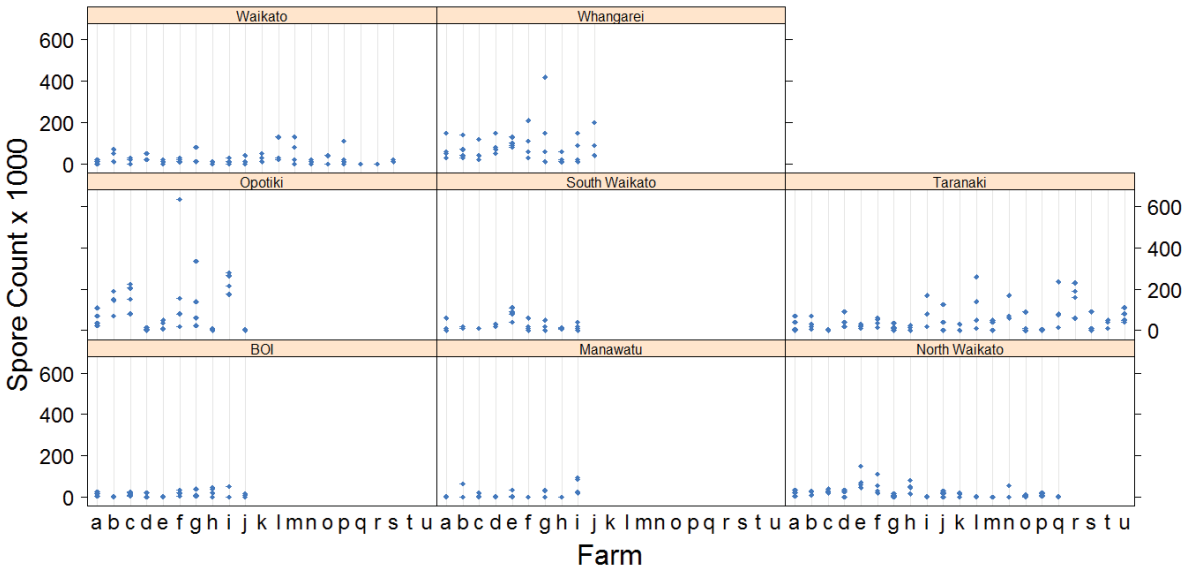


*Figure 6.1: Dot plot of spore counts in each individual paddock (blue dot) of each individual farm (alphabet letter) in each region.*

Common reasons stated for not monitoring their own pasture spore counts included being too busy, a belief that their own FE counts would not change their decisions about how FE would be managed, a reliance on regional spore counts or spore counts measured on neighbouring properties, lack of familiarity with the technique, and a belief that spore count results were too variable.

**Table 6.1. Number of farms using different management methods for the prevention of FE in different regions**.

| Region | Farms | No Treatments | Fungicide | Drench | Feed | Combo | Water |
|---|---|---|---|---|---|---|---|
| BOI | 10 | 1 | 0 | 0 | 1 | 2 | 6 |
| Whangarei | 10 | 4 | 0 | 0 | 2 | 1 | 3 |
| North Waikato | 15 | 1 | 0 | 1 | 2 | 5 | 6 |
| Waikato | 20 | 0 | 4 | 3 | 3 | 4 | 6 |
| South Waikato | 10 | 0 | 0 | 1 | 0 | 1 | 8 |
| Taranaki | 20 | 1 | 1 | 2 | 2 | 3 | 11 |
| Opotiki | 10 | 0 | 1 | 0 | 0 | 2 | 7 |
| Manawatu | 10 | 1 | 0 | 0 | 3 | 0 | 6 |
| Total | 105 | 8 | 6 | 7 | 13 | 18 | 53 |

Of the 107 herd managers, 98 (92%; 95%CI 85.5-96.6) reported that they had some form of management program in place for prevention of FE (Figures 6.2 and 6.3 & Table 6.1).

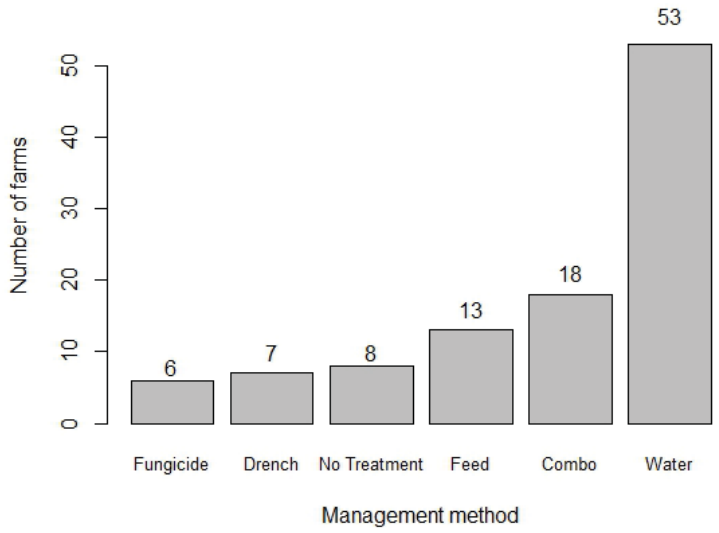


*Figure 6.2: Bar plot of the number of farms in the survey using different management methods for the prevention of facial eczema.*

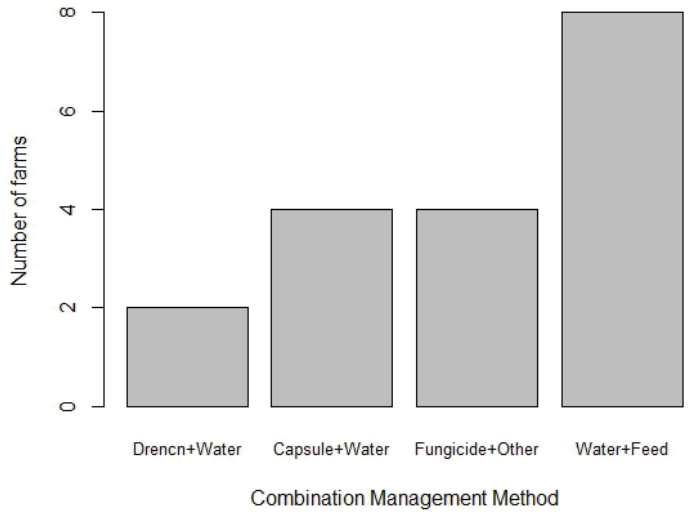


*Figure 6.3: Bar plot of the different combination treatments used for the prevention of FE*

***Fungicide***

This method was used on its own or in combination with zinc treatment on 10 farms. Half of the herd managers using this method used zinc treatment as well. Only two herd managers said they spore counted prior to spraying and three herd managers used spore counts to decide when to stop spraying. Only two herd managers suggested that there were conditions, such as high spore counts or dry, not actively growing pasture, which would stop them from using this method.

***Zinc in water trough***

Of the 68 herd managers who supplemented zinc via the water, 48 used a Dosatron and 6 used a PETA dispenser. The remaining 14 used a variety of techniques including zinc socks, zinc bricks, injector pumps, manually adding zinc to individual troughs and manually adding zinc to main water storage tanks.

Forty-one of the 68 herd managers used some form of flavouring in the water (60%; 95% CI 48-72). Seventeen of 68 herd managers (25%; 95% CI 15-37) said that their cattle had access to other non-supplemented water sources on the farm.

Only 41 of the 68 herd managers (60%; 95% CI 48-72) primed their troughs with zinc prior to spore counts rising by either starting the dosatron early in the season or manually adding in zinc to prime the trough before the cows drank the water.

Forty-seven of the 68 herd managers (69%; 95%CI 57-80) had other stock grazing on the property which had access to the zinc-supplemented water. This included calves, heifers, bulls and beef stock.

***Delivery of zinc by oral drenching***

Nine herd managers used drenching either as the sole method of zinc supplementation or as a combination treatment. Four herd managers drenched every day and five drenched every 2-3 days. Two herd managers did not drench on the weekends.

***Zinc in-feed***

This delivery method was used by 26 herd managers (24%; 95%CI 16.9-34.1) either as the only method of giving zinc or in combination with other methods. Twenty-two out of the 26 herd managers used in-shed feeders (12) or feed pads (10) and the remaining herd managers used more unconventional methods such as mineral blocks, spraying zinc onto the pasture and putting zinc into feed fed out on pasture.

***Zinc boluses***

There were no farmers that used this as the primary and only method for facial eczema control, however there were 8 farmers (7.4%; 95%CI 3.3-14.2) who use boluses as a secondary method in certain situations such as in cows that were dried off early, certain animals that were known not to eat meal or carry over animals.

***Zinc dose rates***

For water-based supplementation zinc dose rates per cow were highly variable (Table 6.2&6.3). Additionally, only 19/68 herd managers using water treatment maintained the same dose of zinc

throughout the season. For example, some herd managers started the FE season using a half dose and when spore counts increased used a full dose.

Drenching dose rates per cow were also highly variable with a range of 5.7g/cow/day to 32g/cow/day. While there should have been different dose rates, and in particular increasing dose rates with lower frequencies of drenching, this trend was not apparent and there were farms that had higher per cow dose rates that drenched daily than those drenching every third day.

Dose rates for zinc in the feed were probably not an accurate assumption of what was being administered. Eight of the ten farmers using zinc with feed on the feed pad gave the kg of zinc used daily which gave a dose range of 4g/cow/day to 40g/cow/day but there seemed to be a general confusion with answering this question and overall a lack of specificity when providing the quantity used. For those farmers who provided zinc through in- shed feeding systems, there was a general assumption that the feed company provided enough zinc to treat a cow of a particular average weight but very little understanding on what the feed companies were putting in and how many grams that would equate to per cow.

Data were available from 68 farms that administered zinc in the water or as a drench on the number of animals being treated daily with zinc and the kilograms of zinc being used per week. The dose rates for the different types of zinc were calculated (Farm Guard reference manual, Ixom Operations Pty Ltd, Australia) and on 42/68 farms (62%; 95% CI 49-73) the daily dose of zinc per cow calculated from these data would not be sufficient to treat a 400 kg cow. Four of the 68 farms were feeding zinc at concentrations in excess of what would be used to treat a 550kg cow. Three of these farms were those supplying zinc in the feed and one of them was zinc through the water. The farm that was over supplying zinc in the water only had two out of the 10 sampled cows with an adequate concentration of zinc in the blood. As a contrast two of the farms oversupplying zinc in the feed had all cows with zinc concentrations above 20µmol/L but also had cows with zinc concentrations well into toxic levels with one farm in particular having cows with a range from 61 to 170µmol/L.

From the questionnaire farmers were not only asked directly how many cows they were treating and how many kg of product they used weekly, but were also asked earlier in the questionnaire, questions about all the animals they had on their property (ie, calves, beef animals, heifers).

Using the number of total animals to be treated on the property in comparison to the number of animals that the herd managers stated they were treating later on in the survey, the dose rates using the different number of animals provided were calculated and the dose rate and discrepancy between the two is shown in figure 6.4.

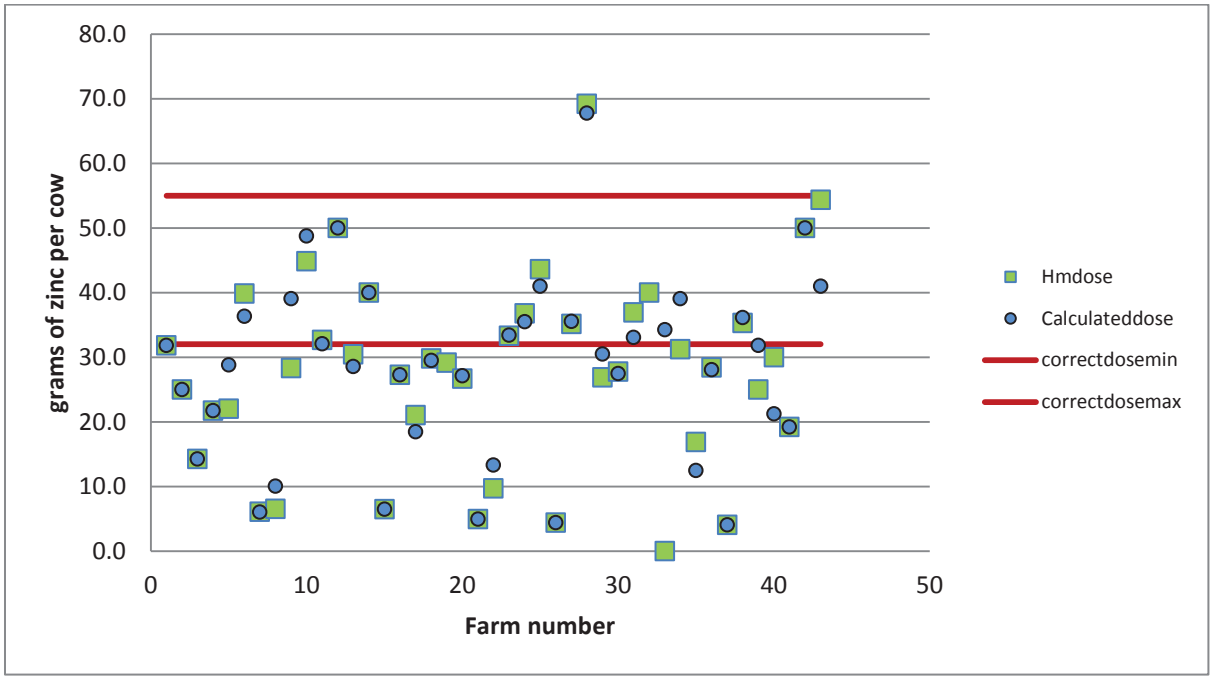


*Figure 6.4: Calculated dose rates that herd managers advised they were providing to cattle daily (Hmdose) and those calculated using information of stock numbers provided earlier in the survey for herd managers that use zinc heptahydrate through the water (Calculated dose). Red lines indicate the correct dose required for a cow weighing from 400 to 550kg.*

***Cattle weights***

Only five farmers weighed their cattle prior to the start of the FE season; thus on most farms zinc dose rates were not tailored to the calculated average weight of the cattle on the farm. The live weights of cattle within individual farms were also highly variable. Mean live weights and ranges are shown in Table 6.2 and 6.3 for farms which used water-based supplementation and feed supplementation respectively.

**Table 6.2. Cattle weights from farms using water treatment and dose rates calculated from herd manager estimates of daily zinc use.**

| Region | Average weight of cows treated | Average (min-max) range of weight of cows on each farm | No. of farms who weigh their cattle prior to starting zinc treatment | Zinc sulphate monohydrate Average (min-max) dose given to cattle (g/day) | Zinc sulphate heptahydrate: Average (min-max) dose given to cattle (g/day) | No. of farms that use the same dose for the whole season |
|---|---|---|---|---|---|---|
| BOI | 445 | 148 (113-192) | 0 | - | 26g (6-40) | 3 |
| Whangarei | 435 | 129 (85-159) | 1 | - | - | 1 |
| North Waikato | 476 | 184 (117-284) | 0 | 20g | 28g (4-40) | 2 |
| Waikato | 464 | 156 (86-285) | 1 | - | 28g (6.5-50) | 2 |
| South Waikato | 500 | 160 (50-218) | 0 | - | 32g (4-69) | 1 |
| Taranaki | 463 | 144 (71-196) | 0 | 16g | 33g (27-37) | 3 |
| Opotiki | 464 | 164 (71-196) | 1 | 0 | 23g (6.5-32) | 2 |
| Manawatu | 525 | 184 (130-226) | 0 | 28g | 17g (9-28) | 3 |
| Total | 474 | 158 | 3 | - | - | 19 |

**Table 6.3. Weights and dosing of cattle for farms using feed treatment.**

| Region | Average weight of cows treated | Average (min-max) range of weight (Vermunt *et al.*) of cows on each farm | No. of farms who weigh their cattle prior to starting zinc treatment | No. of farms who allow for wastage of feed when calculating dose | No. of farms who said some cows don't eat the feed |
|---|---|---|---|---|---|
| BOI | 505 | 126 (113-140) | 0 | 0 | 1 |
| Whangarei | 520 | 222 (185-264) | 0 | 0 | 1 |
| North Waikato | 511 | 176 (117-258) | 1 | 1 | 4 |
| Waikato | 535 | 176 (107-277) | 0 | 0 | 4 |
| South Waikato | - | - | - | - | - |
| Taranaki | 424 | 159 (112-204) | 0 | 0 | 1 |
| Opotiki | 450 | 152 | 0 | 0 | 1 |
| Manawatu | 536 | 242 (180-385) | 0 | 1 | 0 |
| Total | 503 | 179 | 1 | 2 | 12 |

***Efficacy of zinc supplementation***

Of the 1071 sampled cows, 911 were being treated with zinc and had serum zinc concentrations tested. Of these cows, only 288 (32%; 95% CI 28.6 – 34.7) had serum zinc concentrations within the protective range (Figure 6.5); 32/911 (3.5%; 95%CI 2.4-4.1) had serum zinc concentrations >35 µmol/L (above the recommended range), while 623/911 (68.3%; 95%CI 65.2-71.3) had values <20 µmol/L (below the recommended range and therefore not protective against FE).
Seventy-eight herds out of the 93 that used zinc as a management method (84%; 95%CI 75-91) had more than three cows with inadequate zinc concentration in the blood.

In comparison to drenching, farms which supplemented zinc via a water-based method had a higher likelihood of being a farm with inadequate FE protection (≥3 cows with serum zinc concentrations <20 µmol/L) (Table 6.4; OR 12.75; 95% CI 2.6-69.4).

**Table 6.4. Logistic regression model of factors associated with the odds of being a farm with inadequate FE protection**

| Variable | Regression coefficient (SE) | p | OR (95%CI) |
|---|---|---|---|
| Drench | *Reference* | - | - |
| Zinc in feed | 1.522 (0.935) | 0.103 | 4.58 (0.78-32.70) |
| Zinc in water | 2.546 (0.818) | 0.002 | 12.75 (2.63-69.43)[a] |
| Other | 1.833 (1.025) | 0.074 | 6.25 (0.93-58.92) |

a. Interpretation: In comparison to drench, farms that delivered zinc in the water were 12.75 (95%CI 2.63-69.43) times more likely to have inadequate FE protection.

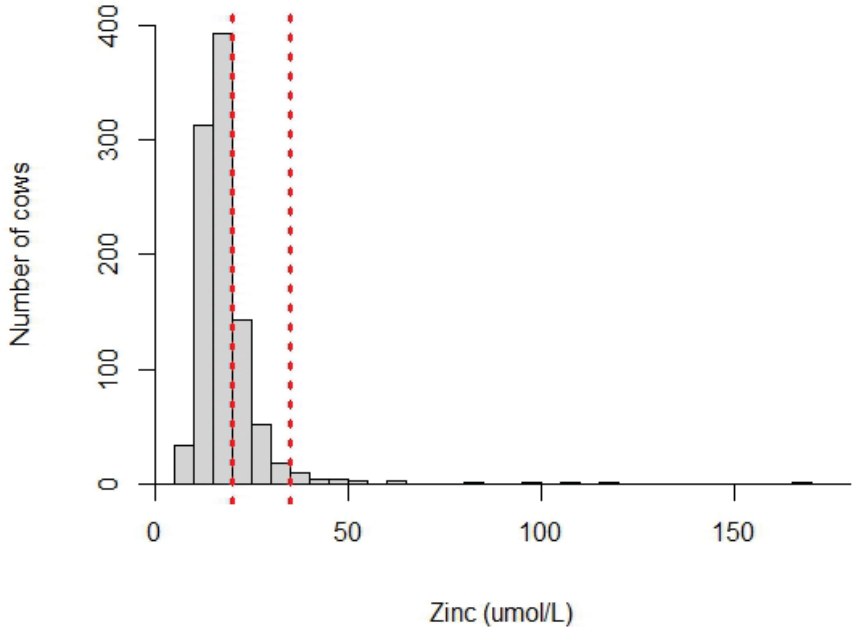


*Figure 6.5: Frequency histogram of serum zinc concentrations in cattle on farms that used zinc to control FE (n = 911). The red vertical dashed lines represent the lower and upper limits of the recommended range for FE protection* (20 to 35 µmol/L).

***Liver damage***

Of the 1071 cows tested 79 cows (7.3%; 95%CI 5.8-9.0) had evidence of moderate to severe liver damage (Figures 6.6 and 6.7), while 35/106 farms (33%;95%CI 24.2-42.8) had one cow or more out of 10 with GGT activity > 300 IU/L (suggestive of liver damage due to sporidesmin challenge). Six of these farms had ≥5/10 cows with elevated GGT activity. The remaining 29 farms had between one and three cows affected. There was no evidence of a difference between regions in the proportion of herds with at least one cow with elevated GGT (p=0.87) (Figure 6.8).

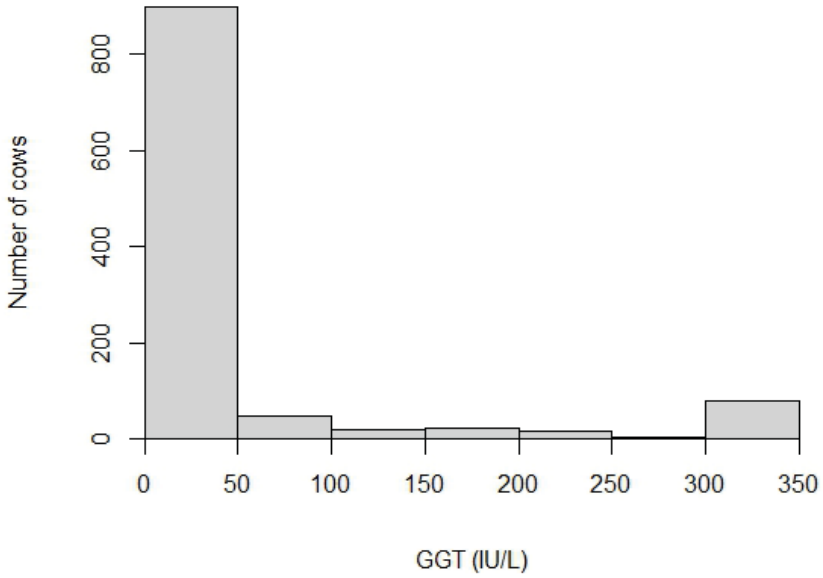


*Figure 6.6: Frequency histogram showing GGT concentrations (expressed as IU/L) for the n = 1081 cows that took part in the study.*

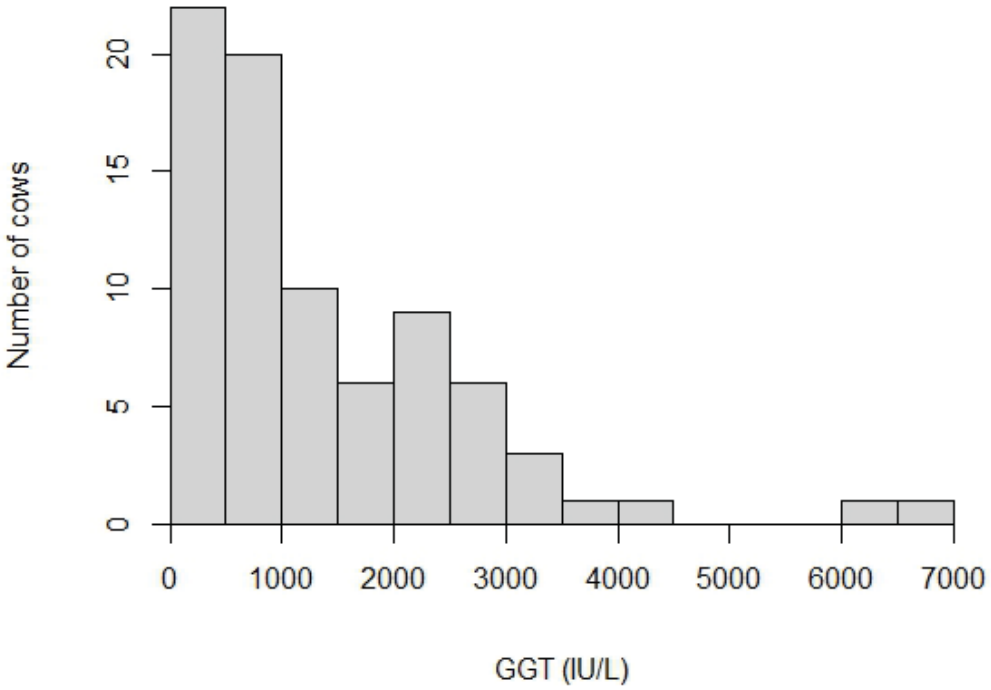


*Figure 6.7: Frequency histogram showing GGT concentrations (expressed as IU/L) for the n = 80 cows with GGT serum concentrations above 300 IU/L.*

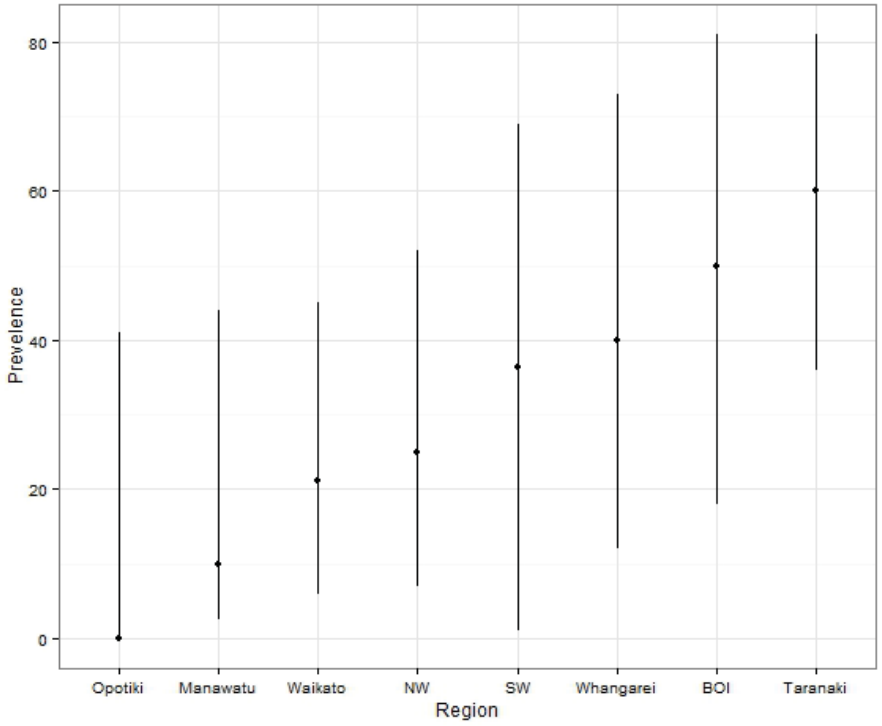


*Figure 6.8: Error bar plot showing the regional individual cow prevalence of FE (with their 95% confidence intervals).*

***Farmer perception on the effectiveness of their FE management program***

Of the 104 herd managers that responded to the question, 72 thought that their FE management program was effective (69%; 95%CI 59-78). Of the 35 herd managers who had evidence of liver damage in their herds, 20 thought their FE management program was effective (57%; 90% CI 39-74). Herd managers with at least one cow with elevated GGT activity were less likely to consider their FE management to be effective than those with no cows; this was almost significant at the 5% level (OR 0.44; 95%CI 0.18-1.04)

Of the 72 herd managers who thought that their FE management program was effective, 67 based this conclusion on not seeing any cows with clinical signs or having FE related deaths in their herd. Of the ten managers who thought their management was not effective their assessment was based on them having a history of or current clinical signs in the herd.

***Young stock***

Of the 97 herd managers that responded to the question, 73 (75%; 95% CI 65 - 83) reported that they had some form of management program in place for their young stock (Figure 6.9.)

A total of 57/73 (78%; 95% CI 66.8 – 86.9%) of herd managers thought their management program for young stock was effective. For all herd managers this conclusion was based on an absence of clinical signs in their young stock. Only 6/97 (6.2%; 95% CI 2.3 - 13%) of herd managers reported that they had tested their young stock for sub clinical FE previously.

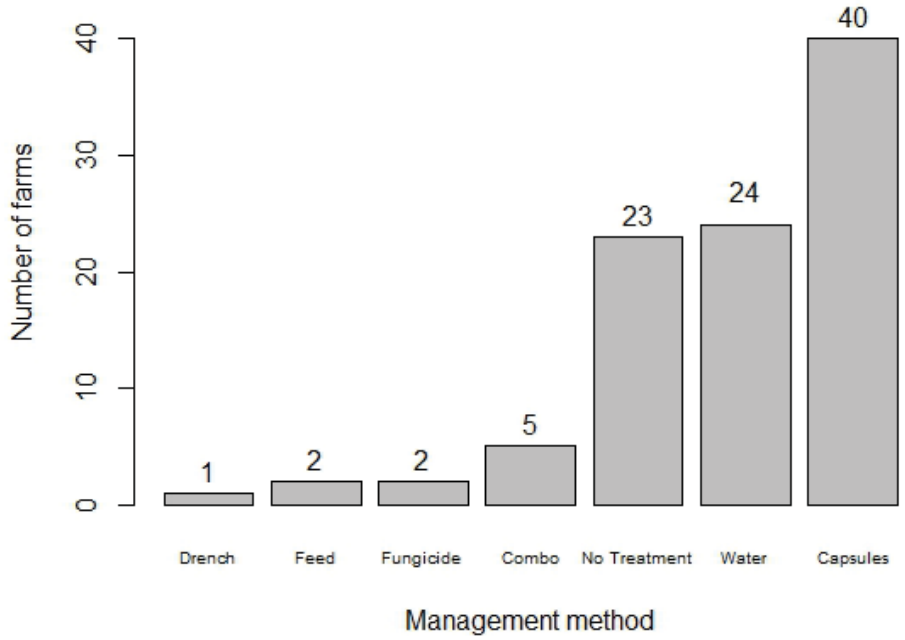


*Figure 6.9: Bar plot of the number of farms using each of the different management options for the prevention of FE in young stock (n=97).*

## 6.5 DISCUSSION

This study showed that FE is not managed effectively on many dairy farms. One third of the farms tested had evidence of elevated GGT activities which was likely to be due to facial eczema damage and only 32% of cows in herds with an FE management program using zinc had serum zinc concentrations within the recommended protective range.

The likely reasons for this high proportion of affected herds and insufficient zinc concentrations can be grouped into 5 categories: 1) timing of management programmes; 2) inadequate dose rates; 3)

inadequate knowledge of management methods; 4) inadequate understanding of the disease; and 5) lack of a consistent message from veterinarians and rural professionals.

***Timing***

Spore counting is the only tool available that can help herd managers visualise trends in the FE risk. Spore counts can be highly variable between and within farms, but if used to monitor trends on individual farms, spore counting can identify when to start and finish FE control programmes. This is because when spore counts are relatively low (such as is the case for the triggers to start and finish control programmes) the variability is much less than when the spore counts are high (Cuttance *et al*. 2016 *unpublished*). Despite this, individual farm spore counting as a tool is under-utilised with only one third of farms monitoring their own spore counts and <5% monitoring the same paddock regularly as is recommended (Marbrook and Matthews). The lack of knowledge of FE risk at the individual farm level means that many herd managers may be starting their FE management programme too late (increasing the risk of liver damage) or too early (increasing the costs of control and the risk of zinc-related toxicity). This is an even bigger concern where herd managers are using fungicides, as fungicide treatment of pastures when spore counts are still low is essential if effective control of FE is to be achieved.

***Incorrect dose***

Inadequate zinc intake is the only plausible reason as to why zinc concentrations are low in cows which are being supplemented prophylactically with zinc to prevent FE. In many cases this is likely to be due to cattle being administered inadequate doses of zinc. This study has identified several reasons why this is the case. Firstly, many farms are not feeding the right dose of zinc; 32/60 farms with sufficient data to calculate the zinc dose being given were not giving enough zinc per cow to prevent FE in a 400 kg cow. Such under- dosing is likely to result from incorrect estimates of zinc requirements, incorrect estimates of the number of cows being treated, or to errors in converting from cow level to herd level zinc requirements.

Errors in the amount of zinc being drenched (due to not accurately measuring the daily dose) could also result in reduced intakes. A possible error when drenching cattle is to not take into account dosing frequency of less than daily; 20% of the herds which used drenching did not drench at the weekend. In such cases the dose rate needs to be increased to compensate, otherwise the amount of zinc available is reduced.

Incorrect live weight is a large potential source of error. Only 5 herd managers weighed their cattle prior to the FE season, so most farmers were dosing based on estimated live weight. The natural tendency for this to result in under dosing is exacerbated by the fact that even dosing to average weight will lead to under dosing 50% of the herd. In this study there were large differences between weights in cattle on each farm (up to 385kg); ideally, cattle should be dosed based on their individual body weight. This is feasible for farms which use individual treatments such as drenching or boluses, but not for farms which use methods which involve the cow having control of its own intake of zinc (i.e. feed and water-based methods). Such methods will, inherently, result in a higher variation of zinc intake. This is partly because, although cows with a higher body weight will tend to eat and drink more than smaller cows, this association is subject to significant individual variation. For example, zinc intake from water-based systems can vary significantly due to weather, stage of lactation, dry-matter content of feed, and cow hierarchy (Castle and Watson 1973; Murphy *et al.* 1983). Taste of the supplemented water can also affect intake, particularly if other sources of water are available. Intake of zinc from supplemented feed will alter for similar reasons (Smith 1980). Farms which use feed or water-based zinc supplementation need to pay particular attention to ensuring that the amount of zinc used per cow per day is at least sufficient to treat the average bodyweight of the cows in the herd and to regularly monitor zinc intake to ensure that at least 8/10 cows have serum zinc concentrations within the recommended range.

Zinc dose rates for cows supplemented by feed have similar challenges to that of water. The different feed intake of cows, particularly when supplemented on the feed pad, alters the intake of zinc. Further challenges to this system could be how it is mixed in with the feed. Different

commercial feed suppliers and farmers have different types of mixing options. Zinc can be incorporated into pelleted feed or added as a powder around the feed. Zinc can also be manually added to a mixer wagon or a feed out wagons by the farmer. While this study did not look specifically into the variability associated with zinc administration into the feed there would undoubtedly be places where variability and error would occur. This was demonstrated in the blood results from one farm being highly toxic (61-170µmol/L) which was caused by a calculation error from the herd manager.

***Inadequate understanding of particular management methods***

Inadequate zinc intakes can also result from failure to effectively apply a control method. For example, of the herd managers that used water-based zinc supplementation, only 60% primed their troughs with zinc prior to spore counts rising in their area. For those herd managers, particularly those using a Dosatron, the failure to use trough priming means that cows would not be protected until the replacement water has completely replaced the water that was initially in the trough when zinc supplementation began. Depending on the grazing rotation, cows can graze for a month before receiving an adequate dose of zinc which may be far too late to protect against the sporidesmin toxin.

Minimising the access of supplemented cattle to non-supplemented sources of water is crucial for maintaining consistent zinc intakes. Despite this, 17/68 farms which used water-based supplementation allowed treated stock access to non-supplemented water.

Another potential cause of under dosing is the impact of evaporation from other troughs on the amount of zinc being directed to the trough where cattle are grazing and the amount distributed to other evaporating troughs.

Similarly, there is a lack of understanding on the effect of dosing non-lactating stock as well as lactating stock from the same dispenser when drinking rates differ markedly when stock are dry versus lactating.

Young stock need a higher concentration than cows as they drink less/kg body weight, therefore water-based prevention of FE maybe effective in lactating cows but not in young stock. Increasing

the concentration of zinc to levels sufficient to prevent disease in young stock is not feasible if adult cows are also using the same water supply as it will significantly increase costs, result in overdosing of cows if water intakes are maintained and increase the risk of cows not drinking due to water palatability issues. More information is required on how to manage water-based zinc supplementation when different stock types are being supplied from the same water source.

Of particular concern, based on the results of this survey, was the inadequate knowledge surrounding the use of fungicide. Firstly, while fungicides have been proven to work, to be effective they need to be used prior to sporidesmin production; i.e. when spore counts are still low. This is because while fungicides may have a small effect in reducing existing spore counts, their primary mode of action is to kill the fungus (Parle and di Menna 1972b, 1972a). Therefore, if fungicides are used on high spore count pastures, cows subsequently grazing those pastures could still get FE damage from the pre-existing spores. Secondly, to be effective the fungicide needs to be taken up by the grass as it is growing (Marbrook and Matthews). If grass growth has markedly slowed or stopped as it does in many parts of the upper North Island during the summer, the fungicide will not work. Finally, fungicide application across the whole farm is essential; in particular fungicide needs to be sprayed under trees and alongside hedges, areas where helicopter application will not reach.

Farmer understanding of these three main features was poor. Instead of correctly applying fungicide as a sole method of controlling FE, many herd managers using fungicide used it alongside low doses of zinc. This means that if the fungicide is effective, cattle are being fed unnecessary zinc, but if the fungicide is not effective then the low dose of zinc is unlikely to provide effective prophylaxis against FE.

***Inadequate understanding of the disease***

This survey has highlighted a major disparity between whether a farm has damage resulting from sporidesmin intoxication and the farmer's perception of the effectiveness of their FE management programme. This is because most herd managers in this survey thought of FE as a clinical skin disease and thus perceived their management programmes to be effective when clinical signs were

not present (or stopped). There needs to be a real effort from veterinarians and rural professionals to work on re-education of herd managers, to highlight the importance of the subclinical liver damage and to focus prevention efforts on controlling liver damage rather than clinical FE

***Lack of a consistent message from veterinarians and rural professionals***

Over the past 60 years there have been many theories on the control of facial eczema. Herd managers have all had different experiences and so have veterinarians and rural professionals. The result of this is that there are a lot of widespread inaccurate myths about control of the disease and very varied advice and information. To make a difference to the management of this disease, those giving out advice need to base their advice on proven evidence-based programmes, not on anecdotal data from farm cases. This is the only way to ensure that the information herd managers are getting is consistent and effective. A recommended protocol for facial eczema management based on peer reviewal is presented in figure 6.10 (references contributing to the protocol development are presented as supplementary material).

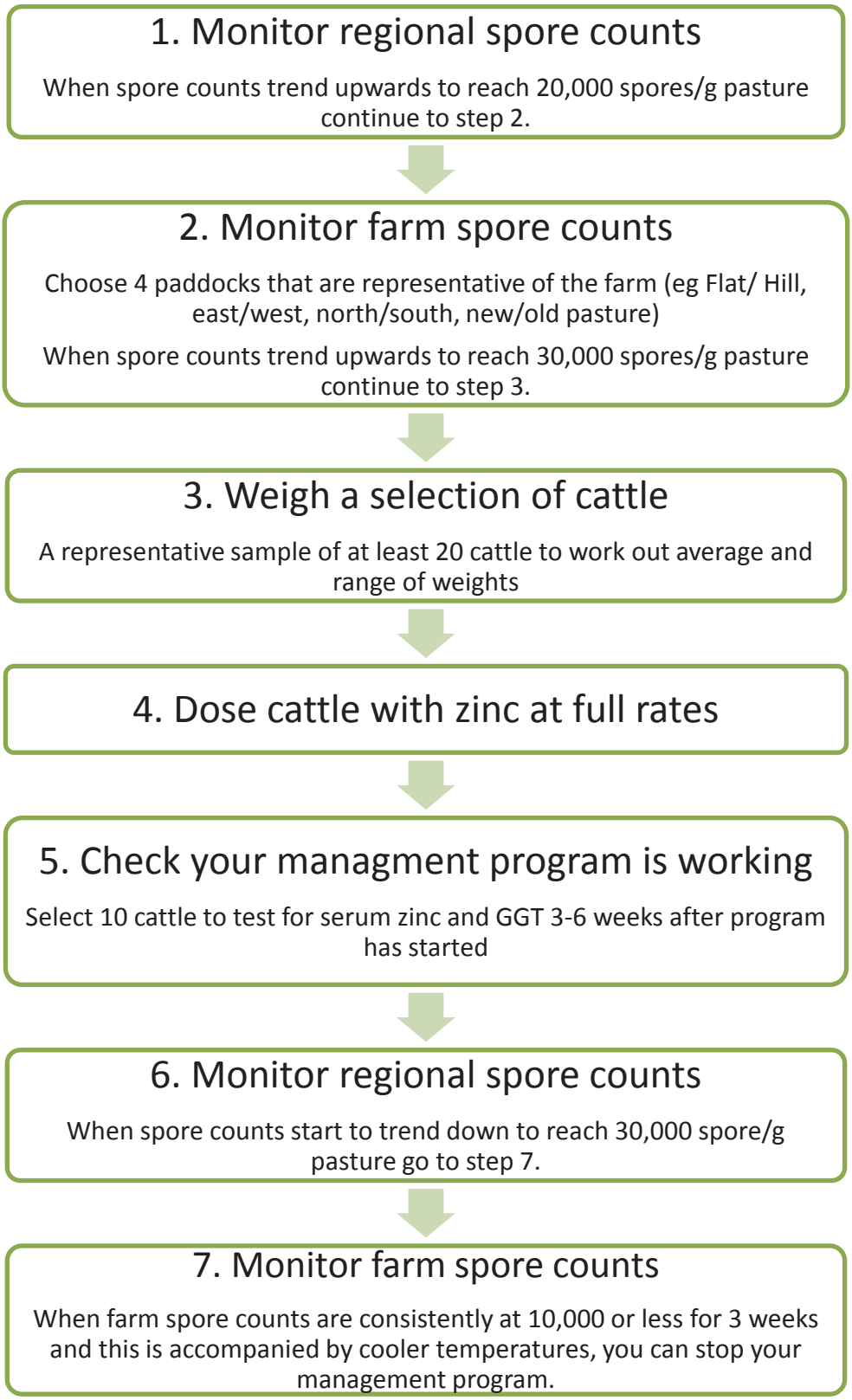


*Figure 6.10: Protocol for managing facial eczema based on peer review research.*

## 6.6 CONCLUSION

This survey has shown that even though all the herd managers in this survey were aware of FE and almost all herd managers implemented some form of FE control, too many farms had ineffective control of FE (elevated GGT) and for farms using zinc supplementation, too many had insufficient zinc intake. This study has highlighted that FE management on dairy farms in New Zealand could be substantially improved; principally through farmers getting more information on the success of their FE management programmes and responding when tests show that FE management is not effective.

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# Supplementary Material

## References contributing to developed protocol (figure 6.10)

***Predicting danger periods by spore counting***


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