is not widespread in the sheep industry and not used at all in the dairy industry. Identifying the genes controlling resistance to FE and developing gene marker assisted selection procedures represents a major opportunity. The same or similar gene markers may operate in sheep, cattle and deer, so that identifying genes in one species could lead to rapid identification of key genes in other species. Phua *et al.* (1999), in a programme now funded by Ovita, identified catalase as a candidate gene having a statistically significant association with FE resistance, but Hohenboken *et al*. (2004) concluded recently that catalase, alternate forms of superoxide dismutase-1 (cytosolic or mitochondrial), glutathione peroxidase-1 and glutathione reductase, played only minor roles in determining genetic differences in FE resistance in sheep. Further DNA studies in sheep are continuing (e.g., Duncan *et al*., 2002, 2005). Where divergent breeding lines have been established, this approach could be applied to other mycotoxicoses such as ryegrass staggers. The opportunities which these lines provide should not be squandered. A major cost in carrying out DNA-marker searches for disease resistance is the cost of scoring/ranking large numbers of animals for resistance. The procedure described by Cullen *et al.* (2006), using data from dairy sire proving schemes following outbreaks of the FE disease, could have application in the sheep industry and to diseases other than FE.

Review of zearalenone studies with sheep in New Zealand

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ABSTRACT

Zearalenone is a naturally occurring mycotoxin from the *Fusarium* fungus which grows on pastures in New Zealand in autumn, and it has been found on farms in some years from Northland to Southland. The toxin may interfere with oestrogen-related functions in sheep during reproduction, reducing ovulation rates and fertility and thus lambing percentages, because its chemical structure is similar to that of reproductive steroids. Forty four per cent of over 6000 New Zealand pasture samples, collected in autumn and tested for zearalenone, were found to have high enough levels for ewe fertility to be either depressed (9% of samples), or 'at risk' (35%). Control of zearalenone toxin production or of *Fusarium* growth on pasture on a large scale is currently not feasible. Attempts to mitigate its effects by immunisation have failed or even exacerbated the problem. Provision of alternative zearalenone-free feed crops is costly and generally uneconomic. Selection of sheep for genetic resistance would seem to be the most beneficial approach. Resistance to zearalenone is inherited in sheep (heritability estimate = 0.32 ± 0.10), and a test could be set up in ram-breeding flocks to select for resistance.

Keywords: zearalenone; sheep; reproduction; genetics; resistance.

INTRODUCTION

Zearalenone is a naturally occurring mycotoxin from the *Fusarium* fungus which grows on moist, dead plant material in many New Zealand pastures in autumn. In survey work, Garthwaite *et al.* (1994) found zearalenone at toxic levels in autumn on at least some pastures throughout New Zealand from Northland to Southland. Nine per cent of over 6000 samples tested had zearalenone at high enough levels for ewe fertility to be depressed, and another 35% were from paddocks where flocks would be 'at risk'. Although it is a mycotoxin, the chemical structure of zearalenone is unrelated to that of the facial eczema-causing toxin, sporidesmin. Instead, its structure and its metabolic breakdown products are similar to that of the reproductive steroid hormones. This enables it to bind to the oestrogen receptors of mammals (Coulombe, 1993), interfering with the signal transduction and control functions of endogenous oestrogens. In adult sheep, the primary effect of zearalenone is to reduce ovulation rate and pregnancy percentage, resulting in decreased lamb production (Smith *et al.*, 1986, 1987a).

Weather patterns conducive to zearalenone production are not particularly restrictive (Towers, 1996), and there appears to be no close relation of pasture zearalenone concentrations with a range of climatic parameters, so that predicting the likely presence or severity of zearalenone is difficult. Unlike the fungus responsible for facial eczema, there is no relationship between *Fusarium* fungal spore counts and toxin (zearalenone) concentration. Peak spore counts tend to precede peak zearalenone concentrations in pasture, and pasture samples from the same area vary markedly in toxin levels. In addition, because zearalenone is primarily found on dead material, whereas sheep selectively graze green material, the use of pasture measurements are of limited value in determining the extent of the problem in a flock. Measurement of zearalenone levels in blood or urine are much better guides (Sprosen *et al*., 1995). However, the half-life of zearalenone in the blood is relatively short, with levels being almost undetectable within 6 hours of administration, except at very high doses (Smith *et al*., 1992b). Zearalenone and its metabolites were first detected in sheep urine by Plasencia *et al.* (1990). Towers & Sprosen (1992) described analyses of zearalenone in pasture samples and zearalenone in urinary breakdown products (collected from 53 widely separated farms, selected with a history of ewe infertility problems), and they were the first to provide solid evidence for zearalenone as a *widespread* "cause of poor reproductive performance in free grazing animals not receiving grain or grain-based supplements."

EFFECTS OF ZEARALENONE ON REPRODUCTION

Interest in the role that zearalenone has on reproduction in New Zealand sheep flocks grazing pasture was stimulated by the proposal that some substance, 'Factor G', in pasture was responsible for losses in reproductive potential that could not be explained by facial eczema (Jagusch *et al*., 1986).

A series of experiments at Ruakura (Smith *et al.,* 1986, 1987a&b, 1990a&b, 1991a, 1992a) have shown that zearalenone intake by breeding ewes results in a disturbance of their oestrous activity (altered duration of oestrus and shortened cycle length or complete failure to cycle), reduced ovulation rate and reduced fertilisation of those eggs ovulated, all contributing to lowered lamb production. These physiological responses are supported by endocrinological data with ewes exposed to zearalenone showing depressed circulating levels of follicular stimulating hormone (FSH) on days 9 to 14 of the cycle (Smith *et al*., 1987b) and the extent and duration of depression in FSH levels are dependent on the dose of zearalenone received (Smith *et al*., 1992b).

The extent of the effect and the duration of zearalenone's influence on ewe reproductive performance depend on both the quantity of zearalenone ingested and the period of time during which it is ingested, with the higher doses and/or longer durations exerting the greatest depression in reproductive performance. A feeding period of as short as 5 days at a level of 6 mg/ewe/day significantly reduced ovulation rates and the effects of as little as 1 mg/ewe/day for 20 days still resulted in a 20% reduction in ovulation rate and carried over for at least one cycle after zearalenone intake ceased. Prolonged exposure for 20-40 days resulted in the effects persisting for at least two cycles after exposure ceased.

In general the effect is a depression in lambing rate of about 5% for every 1 mg/day of zearalenone ingested in a short period (5-7 days) and about twice this for longer (20-day) periods of exposure. The overall effect is dependent on the time of the breeding season when zearalenone exposure occurs, with high intakes prior to (and during) mating increasing the number of barren ewes, and reducing the incidence of twins in those ewes that lamb. Because of the relatively short-lived nature of the effect (e.g. recovery after about 1 to 2 cycles) exposure to zearalenone may have little effect on the final lambing percentage, but it would be necessary to leave the rams out longer (prolonging the mating period) as ewes will cycle later in the season, and there could be major financial impacts with the extended lambing season resulting in a high proportion of late lambs, smaller at the usual weaning date.

Unlike in pigs, exposure of ewes to zearalenone at high levels (24 mg/ewe/day for 10 days after mating) had no effect on embryo survival and lambing performance (Smith *et al*., 1987a, 1990a), but effects at other stages of pregnancy have not been studied.

The effects of 'long term' or repeated exposure year after year are unknown, but extrapolation from other forms of oestrogen exposure (e.g., to clover phytoestrogens) suggests that the gradual development of permanent infertility in ewes could be expected due to progressive changes in the anatomical structure of their reproductive tracts (Lightfoot *et al*., 1974).

The fertility of rams appears to be unaffected by exposure to zearalenone (Smith *et al*., 1987a), with intakes of 6 mg/ram/day for a period of 60 days having no effect on fertility, as assessed by semen collection and natural mating trials.

TECHNIQUES FOR OVERCOMING THE ZEARALENONE PROBLEM

Immunisation of ewes against zearalenone

It was hypothesised that, if the levels of antibodies to zearalenone in the ewe could be raised by active immunisation, then these would protect the ewe against the effects of zearalenone, by binding the material before it interacted with other oestrogen binding sites. However, trials involving immunogens that markedly increased the circulating zearalenone antibody titres failed to protect against the detrimental effects of zearalenone exposure on reproductive performance, and in fact they exacerbated the response (Smith *et al*., 1991b, 1992a).

Use of androstenedione immunogens (Androvax)

Androvax is known to increase lambing performance in ewes by increasing ovulation rate and it was hypothesised that this immuno-induced increase in ovulations would balance or overcome the reduction in ovulation caused by zearalenone. Treatment with Androvax increased ovulation rate and lamb production, and zearalenone dosing reduced both as expected. However, the combined treatment group (Androvax immunisation plus zearalenone dosing) had the lowest lambing performance, indicating a detrimental interaction of the two treatments, with fewer ewes displaying oestrus and a lower conception rate to the first cycle (Meat New Zealand, 1999b). This negative interaction is similar to that reported for the use of another androstenedione immunogen (Fecundin) in ewes exposed to clover phytoestrogens (Cox *et al*., 1985).

Use of zearalenone-free fodder crops as alternative feeds during mating

It has been demonstrated that, by feeding ewes on alternative crops such as chicory or *Brassica,* the depression in fertility due to zearalenone on pastures can be overcome (Towers, 1996), but attempts to extend these findings to field conditions have proven difficult, with highly variable results in terms of both reproductive performance of the flocks, and the economics of such intervention. This variability is due in part to the unpredictable timing of the natural zearalenone challenge and also the duration of that challenge, and therefore the amount of alternative feed material that needs to be grown or conserved for this purpose (Meat New Zealand, 1999b).

RESISTANCE TESTING METHODS

Whilst studying the effects of zearalenone challenge on ovulation rates (Smith *et al*. (1990a), described above), it was noted that some individual ewes continued to ovulate multiples despite exposure to very high levels of zearalenone. This suggested that certain ewes were either resistant to, or tolerant of, high zearalenone levels and that the problem might be solved through genetic selection for such resistance.

Zearalenone challenge and patterns of zearalenone excretion by sheep

Dosing or grazing systems used for challenge

Responses of sheep at Ruakura to zearalenone have been monitored in order to rank animals for resistance, following a pasture challenge or dosing with zearalenone, as follows: 1). Ewes, and lambs of both sexes, have grazed pastures containing elevated levels of zearalenone in autumn (mid March to mid May) (Morris *et al*., 2005a). 2). Ewes have been dosed with zearalenone (10 mg/head/day) for 6 days in autumn, in successive seasons. Responses of ewes to dosing with zearalenone were monitored as ovulation rate at the next oestrus, and an ovulation rate index (over years) was constructed for each ewe (Morris *et al*., 2005b). Zearalenone and zearalenone breakdown products (Zen) in urine have been analysed following both pasture and dosing challenges, and summarised as Zen/Cr ratios, where Cr = creatinine concentration in urine, a method of making partial adjustment for different volumes of urine in the bladder of each animal sampled. Under research-station conditions, this Zen/Cr ratio has been used for a heritability estimate of response to zearalenone challenge (see below: Morris *et al*., 2005a).

The terms ‘resistant’ and ‘susceptible’, as defined by urinary Zen/Cr ratio, still need clarification with further experimentation, in order to determine whether animal variation in Zen/Cr is principally the result of differences in gastro-intestinal absorption rate, metabolic breakdown rate, partitioning differences between the urinary and biliary routes for excretion, or numbers/affinity of available receptors. The degree of re-cycling of zearalenone and its breakdown products may also be important. Ovulation rate differences are the primary criterion of susceptibility, and urinary Zen/Cr after zearalenone dosing has been suggested as a physiological indicator of this criterion, as it is quicker and cheaper than laparoscopy (lower values of the urinary Zen/Cr ratio being indicative of a better ability to resist

effects of the zearalenone challenge, and associated with higher ovulation rate). An experiment with zearalenone dosing and ovulation recording, where Zen/Cr is monitored in peripheral blood, urine and faeces, would provide further explanation of the underlying biology. One difficulty in sheep is that high dose rates would be required with the present assay methods, in order to obtain detectable levels of immunoreactivity from blood. The time relationship between plasma concentration and toxin intake also needs further investigation: after oral administration of various doses of zearalenone, there was a linear dose-relationship with zearalenone breakdown products in blood samples taken 30 minutes later, but not in samples taken 6 hours after dosing (Smith *et al*., 1992b).

Sex effect

There was a consistent 1.75-fold difference in Zen/Cr ratios between males and females, following a standard dose, with the greater value found in females. One possible contributing factor may be a difference in the number or affinity of oestradiol receptors in males and females, a finding that has been under investigation in sheep, at least in specific tissues, the preoptic area and hypothalamus (Scott *et al*., 2000).

Time course of excretion, up to 50 hours

The time course of zearalenone and zearalenone-breakdown products found in urine, after dosing, has been studied (Morris *et al.*, 2005a). Concentrations at 50 hours after dosing were only 4% of the high levels which occurred at 2 hours after dosing. This work was carried out in hogget ewes at dose rates of 4, 6 and 8 mg zearalenone.

Repeatability of animal ranking

Three small trials have provided the opportunity to follow urinary response (Zen/Cr), at different times after dosing with zearalenone, or following different rounds of dosing. Results have been variable, giving correlations within animals of 0.82 (repeated sampling within trial), and 0.27 and 0.23 (the same animals each monitored in two trials). Additional factors explaining this variability have yet to be identified.

GENETICS OF RESISTANCE

Heritability

The field test measuring response to a pasture challenge or to oral dosing with zearalenone had a heritability of 0.32 ± 0.10, using Ruakura lambs and hoggets. This value was nearly as high as for resistance to facial eczema and ryegrass staggers. If this result is substantiated in further testing in industry flocks, then a challenge test could be offered to industry ram breeders, in a similar way to the present sporidesmin testing service offered by Ramguard (Morris *et al.,* 1994).

Selection line differences

- **Ryegrass Staggers lines:** Dosing of the AgResearch Resistant and Susceptible Ryegrass Staggers (RGS) selection lines of sheep with zearalenone in two separate studies (66 half-bred lambs born in 2003, and 46 selection-line ewe hoggets born in 2004) showed differences among sire groups suggesting that there was a small favourable genetic correlation between resistance to the lolitrem toxin for RGS and the zearalenone toxin (C. A. Morris, unpublished data).

- **Facial Eczema (FE) lines:**

  A). Lambs from the Ruakura FE-Resistant and Susceptible selection lines were challenged with zearalenone on pasture, and compared using the Zen/Cr ratio in urine. The FE-Resistant line tended to have a higher Zen/Cr ratio than the Susceptible line. This could mean higher susceptibility in the FE-Resistant line than in its Susceptible line, but alternatively it could mean an ability to metabolise and excrete toxin faster. (B). Smith *et al*. (1988) examined the impact of zearalenone on ovulation rates of ewes from the FE Resistant and Susceptible selection lines. The Resistant-line ewes had higher ovulation rates than Susceptible-line contemporaries both prior to (1.90 and 1.50, respectively) and following zearalenone challenge (1.35 and 1.22, respectively). Because the percentage decrease in ovulation rate due to zearalenone challenge was non-significantly larger in the Resistant than in the Susceptible line (29 and 19%, respectively), it was concluded that genetic resistance to FE did not confer protection against the effects of zearalenone, but the trend was for the Resistant line to be more susceptible to zearalenone than the Susceptible line. Consistent with these data was the observation that FE Resistant-line lambs grazing pastures with elevated zearalenone levels had 38% higher urinary concentration of zearalenone metabolites than FE Susceptible-line lambs on the same pastures (C. A. Morris, unpublished data). The direction and magnitude of the genetic correlation between resistance to FE and to zearalenone is not confirmed, but the above observations suggest that the relationship might

be antagonistic. FE Resistant-line sheep may have higher activities of Phase I liver detoxification enzymes than FE Susceptible-line sheep (Smith *et al*., 1980; Parkinson, 2001). If these same enzymes are also necessary for the conversion of zearalenone to its breakdown product, α-zearalenol (Liehr *et al*., 1998), then this could be an asset for FE resistance but a penalty for zearalenone resistance, because α-zearalenol is more oestrogenic than zearalenone (Hagler *et al*., 1979; Galtier, 1999), at least in monogastrics. This would contribute to a positive genetic correlation between the breakdown or metabolism of zearalenone and sporidesmin, but a negative genetic correlation between resistance to zearalenone and FE.

Genetic correlation with ovulation rate

There was a genetic correlation of -0.55 between urinary Zen/Cr after dosing, and ovulation rate response (Morris *et al*., 2005b) (i.e. the ewes with lower levels of zearalenone breakdown-products in urine had higher ovulation rates). The genetic correlations between resistance to zearalenone and other production traits need to be worked out, and this should be possible from an industry trial underway in 2005/06, involving eight flocks.

GENERAL CONCLUSIONS

- *Fusarium* and the zearalenone toxin are found distributed on New Zealand pastures nationwide, in some years.
- Zearalenone is a potential cause of severely reduced reproduction in ewes, through interference with levels of both fertility and litter size.
- Its effect on production levels in lambs is not known.
- Control of zearalenone production or of *Fusarium* growth on pasture is not possible on a large scale.
- *Fusarium* and zearalenone are most commonly found between March and May, precisely the sheep mating season,
- The annual economic cost to the nation is unknown, but probably amounts to tens of millions of dollars.
- Resistance to zearalenone is inherited in sheep.
- A simple challenge-test has been developed and could be offered to breeders, using the infrastructure already built up by Ramguard.

Genetic parameters for resistance to facial eczema in dairy cattle

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ABSTRACT

A project was designed to progeny-test dairy industry sires for their resistance to facial eczema (FE), as a preliminary step towards identifying DNA markers or genes for FE resistance. The FE disease is caused by the toxin, sporidesmin, produced by spores of a fungus, *Pithomyces chartarum*, found on many pastures in summer and autumn in the North Island of New Zealand. In susceptible animals, sporidesmin causes liver injury, and the cost of FE to the dairy industry is measured in tens of millions of dollars in years with serious outbreaks ($3.6 to 66.2M *per annum*). Earlier studies in New Zealand have established that resistance to FE in cattle is a heritable trait, with resistance measured by variation in activity in blood of liver-derived enzymes, gamma-glutamyltransferase (GGT) and the associated glutamate dehydrogenase (GDH). Widely-used Friesian and Jersey sires were progeny-tested via 572 specially-reared sons (born in 2002-04 and dosed with sporidesmin), and also via 3761 daughters in autumns 2004 and 2005 in 17 herds with 3-22% clinical cases of FE per herd. The data were combined from all sources, including four earlier years of GGT records (1173 animals born in the 1986, 1989, 1990 and 1992 birth years), and standardisation was applied to each contemporary group. Heritabilities were estimated for Friesians (0.47 ± 0.07 for log GGT; 0.32 ± 0.08 for log GDH) and for Jerseys (0.37 ± 0.06 and 0.39 ± 0.09, respectively), and there was a very high genetic correlation between activities of the two enzymes (0.93 ± 0.03). Sixty-eight sires had reliabilities of >0.70 for log GGT Breeding Value and 71 others had reliabilities between 0.60 and 0.70. The sires progeny-tested