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ZEARALENONE AND ITS PRESENCE IN PASTURE

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SUMMARY

The role of zearalenone, a mycoestrogen produced by soil-borne, free-living fungi, was reviewed for its significance to grazing ruminants, and a pilot survey then conducted in south west Victoria. Zearalenone was widespread in dairy, beef and sheep pasture in the survey conducted during autumn-winter. Zearalenone was present in 80% of 87 randomly selected pastures. The maximum concentration observed was 21 mg/kg DM. For 31% of all pastures, zearalenone exceeded 1.0 mg/kg, the level above which overseas research has suggested that fecundity in sheep may be adversely affected. Within this data set, zearalenone concentration was independent of mean annual rainfall, date of sampling, pasture height and pasture age. Investigations into infertility of livestock should take into account the possible role of mycoestrogens. Their possible additive/synergistic effect with mycotoxins is discussed.

Keywords: mycotoxins, oestrogens, zearalenone, zeranol, residues, fecundity, pasture, *Fusarium*

INTRODUCTION

Hyperoestrogenism and reproductive problems in grain fed pigs are associated with grain contamination by saprophytic fungi. Fungal production of metabolites with oestrogenic potency became evident some 70 years ago, particularly with pigs. An oestrogenic secondary metabolite, zearalenone, was eventually identified as a major cause of these problems. Hagler *et al.* (2001) reviewed these discoveries and the risk that zearalenone posed to livestock and the human food chain. Zearalenone, and its major metabolites, α-zearalenol and β-zearalenol, elicits significant oestrogenic activity in animals, and α-zeralenol is 3-4 times more oestrogenic than zearalenone. The conformation of these metabolites is similar to naturally occurring vertebrate hormones such as 17β-oestradiol. Animals most susceptible are those with oestrogen receptors with high affinity for such compounds. The proportions of α- and β-zearalenols formed *in vivo* in the rumen from zearalenone vary with species, but α-zeralenol, predominates in cattle (WL Bryden, pers. comm.).

Zearalenone and its derivatives are produced by several genera of fungi (El Kady *et al.* 1988), including *Fusarium* species, many of which are widespread in Australia (Bryden and Burgess 1993), and are considered the major source of pasture contamination in New Zealand (Sprosen *et al*. 1995). There is widespread global evidence of contamination of grains with mycotoxins (Placinta *et al*. 1999). Zearalenone concentrations were commonly 0.5-5 mg/kg DM in autumn pasture in New Zealand, especially where dead herbage was present (di Menna *et al*. 1987; Towers 1996, Sprossen *et al.* 1995). In New Zealand, *Fusarium* growth was found to be greatest during autumn (Towers and Sprosen 1991, 1993).

In the rat, zearalenone has reduced intake and liveweight gain, and for the male, sperm counts and testes weight were significantly lowered on a diet containing 0.25 mg zearalenone/kg bodyweight/day (Kaliamurthy *et al.* 1997). In pigs, hyperoestrogenism is common when dietary zearalenone exceeds 1 mg/kg (Haschek and Haliburton 1986), and Gaumy *et al.* (2001) advised against feeding young pigs more than 0.2 mg/kg. Zearalenone contaminated pasture has been established as the causal aetiological agent of low fertility in ewes at pasture (Towers and Sprosen 1991, 1993). In controlled feeding experiments, Smith *et al*. (1990) showed that relative to the sow, the ewe was more sensitive to zearalenone. It decreased the ovulation rate and cycle length, and increased the duration of oestrus, but did not affect pregnancy rate or embryonic loss (Smith *et al.* 1992). From feeding trials, Towers (1996) reported that an intake of 1 mg/day for 7-10 days before mating can depress the number of ewes exhibiting oestrus, and reduce ovulation rate. With 40 days exposure to 6 mg/day (eg 2 kg feed with 3 mg/kg zearalenone), the number of ewes exhibiting oestrus was reduced by 60% and ovulation by 40%. Grazing sheep can ingest zearalenone sufficient to impair reproduction; high levels of the urine zearalenone metabolite, zearalenol, were associated with poor lambing percentage (Towers and Sprosen 1991). Zearalenone reduces the conception rate in heifers (Weaver *et al.* 1986; Diekman and

Green 1992; Webber 1994) and zearalenone in hay/silage is associated with cow infertility (Mirocha *et al.* 1968). Kalella and Ettala (1984) observed that a diet of hay containing 10 mg zearalenone/kg DM was associated with embryonic resorption/early abortion of calves at 1-3 months.

Zearalenone is an anabolic agent that may influence mammary development in the young rat and protect the mammary gland from carcinogen-induced malignant transformation (Hilakivi *et al*. 1999). It stimulates growth in cattle and sheep - α-zearalanol or zeranol, the alkane derivative of zearalenone, has been developed as the growth promotant, Ralgro ®. Zeranol is also formed in cattle from the *in vivo* breakdown of ingested zearalenone (Kennedy *et al.* 1998). Up to 13 ng of naturally produced zeranol/mL of urine has been measured in pasture-fed cattle by Erasmuson *et al*. (1994), who also found natural zeranol and its ipimir, taleranol (β-zearalanol), present at physiologically effective levels in horses. *Fusarium* toxins were detected in 32% of 422 bovine bile samples tested in a UK study (Kennedy *et al*. 1998).

A pilot survey was undertaken to ascertain if zearalenone was present in pastures in south west Victoria and, if it was, the frequency of levels that may have economic consequences for breeding livestock.

MATERIALS AND METHODS

Twenty-nine dairy farms were randomly selected from dairy licensees in south west Victoria, and visited between March 16th and April 15th 1999. A further 58 farms (53 beef/sheep; 5 dairy) were similarly selected from tail tag holders in south west Victoria, and visited between April 27th and July 19th 2000. In each year, a number of sample farms were chosen *pro rata* for each local government area, within which individual farms were selected at random. Managers provided information on each pasture and farm. Samples were collected between 1000-1500 h, using stratified sampling to cover 1 paddock, and collecting pasture at 40 randomly selected sites. Paddock selection was on the basis of next days grazing. Pasture samples included dead and green herbage, and were cut at ground level and transported in a freezer. Zearalenone was determined on ground, freeze-dried herbage by HPLC (Vicam 1998; Visconti 1998). Correlations were examined between zearalenone, annual rainfall and other pasture/site characteristics.

RESULTS

The location of farms fell within the 480-1000 mm/year mean rainfall isohyets, and 4 of the selected pastures were irrigated. All pastures contained perennial ryegrass. Details of farms, herds and sampled pastures are summarised in Table 1.

Table 1. Range in pasture description and zearalenone concentration.

Pasture descriptor	Year	Range	Median	Mean	Standard deviation
Age of pasture (years)	1999	0.5-109	10.0	20.4	23.4
	2000	1-100	17.0	23.7	23.1
Perennial ryegrass ground cover	1999	10-80	50.0	46.4	17.9
(%)	2000	5-95	80.0	71.0	23.4
Time since grazed (days)	1999	1-170	10.0	21.8	32.9
Pasture height (cm)	1999	5-28	12.0	12.5	4.9
	2000	2.5-17.5	10.0	9.1	3.7
Zearalenone (mg/kg)	1999	0-21.0	0.74	1.67	3.8
	2000	0-14.1	0.39	1.08	2.3

Zearalenone was detected in 93% of the pastures tested in 1999, and 74% in 2000, and at least for this small data set, was independent of mean annual rainfall, date of sampling, pasture height and pasture age. For 37% (1999) and 27% (2000) of the farms surveyed, zearalenone concentration exceeded 1.0 mg/kg of herbage on a DM basis (Table 2).

DISCUSSION

New Zealand reports show that most pastures have zearalenone concentrations of <5 mg/kg DM, but they can be up to 25 mg/kg. Analysis of urine found >30% of sheep flocks are exposed to zearalenone in amounts likely to lower lambing rate (Sprosen *et al.* 1995). For short term exposure (10-15 days), Towers (1996) suggested that the toxic dose in pasture for ewes was 1 mg/kg. On this basis, for livestock mated in autumn, 31% of the south west Victorian pastures tested may cause a

mycoestrogen-induced delay in breeding and suppression of multiple ovulations. Zearalenone concentrations were similar to those reported from the larger surveys of pasture conducted in New Zealand in 1992 and 1993 (Table 2). A higher frequency of high concentrations may reflect more pastures carrying large amounts of dead herbage (Towers 1996) as is commonly observed in south west Victoria. For cattle, lower levels of zearalenone may be significant. Authorities advise against the use of feed containing >0.2 mg/kg (Brooks 1997) or >10 ppb (Radostits *et al*. 2000). Associated with our survey, testing for perennial ryegrass endophyte (*Neotyphoium lolii*) alkaloids found that 1 in 3 dairy pastures contained the neurotoxin, lolitrem B and the vaso-constrictor, ergovaline, at levels associated with ryegrass staggers and heat stress (Reed *et al.* 2000). Such mycotoxins have a wide range of physiological activity in herbivores and are associated with ill-thrift and lowered milk production. They can decrease reproductive efficiency (Watson *et al.* 1999; Oliver 1997) and importantly, they may have additive effects with zearalenone. A review by D'mello *et al.* (1999) noted that synergistic effects and/or potentiating interactions have been observed with combinations of some mycotoxins.

Table 2. Frequency of zearalenone concentration ranges in pasture in south west Victoria relative to New Zealand surveys (Towers 1996) - proportion of pastures (%) within designated bands of zearalenone concentration.

Zearalenone	New Zealand		South west Victoria	
(mg/kg)	1992	1993	1999	2000
0-0.29	24	8	14	44
0.3-0.9	51	69	47	29
1.0-2.9	22	21	30	19
>3.0	3	2	7	8
No. of pastures tested	372	494	29	58

The different mean zearalenone concentrations in the 2 years studied may be associated with season or a number of other factors. Samples from the 2 years differed in time of year and enterprise type, and represented the southern and northern parts of the region (differing in mean latitude and rainfall). The sample number was small, but widespread prevalence of zearalenone ingestion by cattle was confirmed by recent residue testing (Coulson 2001). The Australian Residues Survey extended its zeranol testing to include zearalenone, and its major metabolites, α -zeralenol, β-zeralenol, α -zearalanol and β-zearalanol, over 30 months from July 1998. Based on the presence of zearalenone and its derivatives, evidence of zeranol administration was extremely low. Half of 884 bovine faecal samples tested positive for zearalenone/other *Fusarium* metabolites (Coulson 2001). For Victoria, zearalenone was more common in the high rainfall districts. In south west Victoria, 40 out of 50 faecal samples from slaughtered cattle were positive for zearalenone and/or its derivatives (P Miller, pers. comm.).

Thain (1966, 1968) described an infertility syndrome accompanied by hyperoestrogenism in dairy cattle in high rainfall districts of Tasmania. Subsequent feeding studies virtually eliminated phytoestrogens as the cause (Braden *et al.* 1971); mycoestrogens were not investigated. Many factors can underlie infertility, and studies into reproductive efficiency should consider mycoestrogens as a possible factor. The potential for economic losses warrants wider investigation. Studies to identify the species most responsible for zearalenone production, and to understand their ecology, may lead to pasture and grazing strategies that will provide a safe feedbase conducive of improved fertility.

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