Effect of ergot alkaloids on liveweight gain and urine lysergol level in ewe hoggets and heifers

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ABSTRACT

Ingestion of ergot alkaloids can reduce live-weight gain (LWG) in sheep and cattle. The objective of this experiment was to determine if the enzyme-linked immunosorbent assay (ELISA) for urinary lysergol level provided an accurate measure of ergot alkaloid dietary intake. Forty hoggets and 60 heifers were fed ryegrass seed containing ergovaline (EV) (0.0 to 75.0 µg EV/kg LW hogget; 0.0 to 35.0 µg EV/kg LW heifer) daily. The feeding trial was repeated after 30 days for the hoggets. Urine lysergol:creatinine ratio increased linearly for hoggets from 1.91 to 5.80 ($P<0.001$) and LWG was linearly suppressed from 110 to 60 g/d ($P<0.01$) and 30 days later, from 35 to -130 g/d ($P<0.05$) for intakes of 0.0 to 75.0 µg EV/kg LW. Heifer urine lysergol:creatinine ratio increased linearly from 1.60 to 5.21 and LWG declined from 1.0 to 0.85 kg/d for intakes of 0.0 to 35.0 µg EV/kg LW ($P<0.05$). Urinary lysergol:creatinine ratio increased by 0.056 ± 0.007 ($P<0.001$, $r^2 = 0.67$) in hoggets and by 0.082 ± 0.006 ($P<0.001$, $r^2 = 0.77$) in heifers, for every 1 µg EV/kg LW consumed. These studies demonstarted a strong relationship between ergot alkaloid dietary intake and urine lysergol:creatinine ratio.

Keywords: ryegrass; endophyte; ergot-alkaloid; ergovaline; live weight-gain; urine; ELISA; lysergol; creatinine; prolactin; ewe-hoggets; sheep; heifers; cattle.

INTRODUCTION

Ergovaline (EV) is the most potent and abundant ergot alkaloid produced by perennial ryegrass (*Lolium perenne*) and tall fescue (*Festuca arundinacea*) infected with wild-type endophyte (Lyons *et al.,* 1986). These endophytes protect their host against a range of invertebrate pests (Fletcher & Piper, 1990) however they are toxic to grazing livestock and can reduce LWG (Hannah *et al.,* 1990). Ergot alkaloids can act as vaso-constrictors (Strickland *et al*., 1996), dopamine agonists (Larson *et al.,* 1994), inhibit prolactin (Fletcher & Easton, 1997) and cause thermoregulatory dysfunction or "heat-stress" (Bluett *et al.,* 2001).

A suitable diagnostic test is required to quantify the impact of ergot alkaloids on livestock performance in New Zealand. EV and most other ergot alkaloids have a common lysergol ring structure which is excreted mostly in the urine (Hill *et al*., 1994). Hill *et al*., (2000) found a good relationship between urine lysergol:creatinine ELISA (murine antibody) and LWG suppression in cattle grazing fescue pastures in warm ambient conditions.

This paper evaluates a urine lysergol creatinine ELISA (ovine antibody) as a predictor of ergot alkaloid ingestion. It also quantifies the impact of ergot alkaloid ingestion on sheep and cattle LWG under New Zealand grazing conditions.

MATERIALS AND METHODS

Experimental design and treatments

Four groups of 10 Coopworth ewe hoggets (mean LW 38.2 kg) were fed ryegrass seed containing different concentrations of EV adjusted for LW (i.e. 0.0, 18.7, 37.5, and 75 µg EV/kg LW) daily for 28 days. The trial was repeated with the same hoggets after 30 days for another 14 days. In both hogget and heifer experiments the range of dietary ergot alkaloid concentrations was achieved by blending different proportions of ryegrass seed containing nil (0 mg/kg DM) and high 30 mg/kg DM hoggets, 27 mg/kg DM heifers concentrations of EV. In all experiments the ergot alkaloids consisted mainly of EV plus other unidentified endophyte alkaloids, but no lolitrem B. The seed had been ground using a stone-mill to achieve maximum absorption of EV from the diet. Before the start of all experiments, animals were pre-conditioned to the routine and seed ration by feeding ground seed that was free of ergot alkaloids. The concentrations of EV in the seed were determined by HPLC at AgResearch Grasslands (Palmerston North) (Baker *et al.*, 1993). With hoggets, the seed was mixed with dilute crude molasses and water to increase palatability, and fed each morning to individually-penned animals. The hoggets grazed on cocksfoot (*Dactylis glomerata*) and white clover (*Trifolium repens*) pasture that was free of ergot alkaloids, for the remainder of the day. Pasture intake was not measured but was estimated at 1.5 kg DM/hogget/day. The overall EV concentration in the

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total diet was estimated to range from 0.0 to 2.0 mg/kg DM. The hogget trial was conducted for 28 days from 15 March to 11 April 2002 at the AgResearch Lincoln Farm, Canterbury. Average maximum and minimum ambient air temperatures for the first 15 days were 22 ^{o}C and 9.5 ^{o}C, and 14.6 ^{o}C and 6.7 ^{o}C for the last 11 days respectively.

Hoggets were weighed at the same time of the day on day 1, 14 and 28, then again 30 days later, on day 1 and 14. Daily LWG (g/d) was calculated. Urine samples were collected on day 14, 23 hours after seed ingestion, using the respiratory occlusion method (Divers, 1992). The hoggets were re-randomised for the second trial.

On day 15, the hoggets were transferred to an enclosed environment for 4 hours, (ambient temperature 35 ^{o}C, relative humidity >65%) respiration rate and body temperature were measured. Blood samples were taken by jugular veni-puncture for determination of plasma prolactin concentration.

Sixty Hereford-Friesian heifers (mean LW 170 kg) were randomly allocated into eight treatment groups and fed varying rates of EV in ryegrass seed (n = 15 for 0, n = 5 for 5, 10, 15, 20, 25, 30 and n = 15 for 35 µg EV/kg LW). The 300g of total ground seed was mixed with 300g "Mooslee" basal diet (Denver Stock Feeds, Palmerston North) and 300ml of dilute crude molasses and water added to increase palatability. This mixed ration was fed each morning to individually-penned heifers. Daily refusals were measured to enable calculations of actual EV intake. The heifers grazed on chicory (*Chicorium intybus*) and white clover (*Trifolium repens*) pasture that was free of ergot alkaloids, for the remainder of the day. Water and barley straw were offered *ad lib.* whilst grazing. Pasture intake was not measured but was estimated to be 5.3 kg DM/heifer/day. The overall EV in diet was estimated to range from 0 to 0.97 mg/kgDM. The experiment was conducted for 42 days from 28 February to 11 April 2003 at the Aorangi AgResearch Station, Manawatu. Average maximum and minimum ambient air temperatures for weeks 1 to 4 were 24.4 °C and 12.8 ^{o}C, and 19.3 °C and 10.3 ^{o}C for weeks 4 to 6 respectively.

Heifers were weighed weekly, at a similar time of day and daily LWG (kg/d) was calculated. Urine samples were collected weekly, 6 hours after treatment, using manual stimulation (Gibbons, 1956). Body temperature was measured with a digital rectal probe thermometer, once during weeks 3, 4, and 5. Blood samples were taken pre-trial and at weeks 1, 3, and 6 by tail veni-puncture for determination of plasma prolactin concentration.

ELISA assay

In all experiments urine was analysed for lysergol at the AgResearch Ruakura Toxinology Laboratory (Hamilton) and the lysergol:creatinine ratio was calculated. Urinary ergot alkaloid metabolites were measured by an indirect competitive ELISA assay (Garthwaite *et al.,* 1994). The antibody used in this assay recognised the lysergol ring present in the metabolites (Toxinology and Food Safety Research Report 1992-1995, AgResearch). Bovine serum albumin (BSA)-lysergol conjugate was adsorbed onto a microtitre plate. Free non-specific binding sites were blocked with a dilute BSA solution before samples and standards were applied to the plate, and incubated in the presence of anti-lysergol specific antibody. Lysergol in the sample or standard competed with the plate-bound lysergol and reduced the amount of antibody binding to the plate. After washing, the amount of plate-bound antibody was determined by the addition of Horseradish peroxidase (HRP)-labelled goat-anti-sheep second antibody followed by a reaction with HRP substrate. The reaction was stopped by the addition of sulphuric acid, and the coloured product measured spectrometrically. The inhibition of colour development indicated reduced binding of the anti-lysergol antibody to the plate coater (BSA-lysergol), as it was removed by binding to lysergol in the samples. Urinary creatinine concentrations were determined and urinary lysergol levels expressed as per unit creatinine (µmol/mol).

Calculations and statistical analysis

Linear regression analyses of responses (LWG, urine lysergol levels, and prolactin concentrations) to ingested EV levels were performed using GenStat 6^{R} for Windows (2002).

RESULTS

Liveweight gain

Hogget LWG declined from 110 to 60 g/d with increasing level of ingested EV (0.0 to 75.0 µg EV/kg LW) ($P<0.01$) (Figure 1a), and 30 days later, from 35 to -130 g/d (0.0 to 75.0 µg EV/kg LW) ($P<0.05$). Hogget LWG declined 0.7 ± 0.2 g/d ($P<0.01$, $r^2=0.19$) and 2.1 ± 0.3 g/d ($P<0.001$, $r^2=0.53$) for each 1 µg EV/kgLW consumed for the first 28 days and 30 days later respectively.

Heifer LWG also declined with increasing level of dietary EV from 1.0 kg/d (0.0 µg EV/kg LW) to 0.85 kg/d (35 µg EV/kg LW) ($P <0.01$) (Figure 1b). Heifer LWG declined 0.005 kg/d for every 1 µg EV/kg LW increase in ingested EV ($P<0.01$) however, this only explained 9% of the variation in LWG. Over the range of 0 to 35 µg EV/kg LW intake, heifer LWG was reduced by 15% compared to a reduction of 27% and 205% in hoggets for the first 28 days and 30 days later respectively.

Urinary lysergol:creatinine ratio

Urinary lysergol:creatinine ratio was closely related to ergot alkaloid intake in both hoggets and heifers. Ewe hogget urine lysergol:creatinine ratio, measured 23 hours after ingestion, increased linearly with increasing dietary levels of EV from 1.91 (0.0 µg EV/kg LW) to 5.80 (75 µg EV/kg LW) (Figure 2). Heifer urine lysergol:creatinine ratio, measured 6 hours after ingestion also increased, from 1.60 (0.0 µg EV/kg LW) to 5.21 (35 µg EV/kg LW) (Figure 2). Urinary lysergol:creatinine ratio increased by 0.056 ± 0.007 ($P<0.001$, $r^2 = 0.67$) in hoggets and by 0.082 ±

0.006(P<0.001, r^2 = 0.77) in heifers for every 1 µg EV/kg LW consumed.

Hogget LWG decreased by 10 ± 3 g/d (P<0.01, r^2=0.18) and heifer LWG by 0.02 kg/d for every 1 unit increase in lysergol:creatinine ratio but the latter was not significant (P=0.41).

FIGURE 1a: Liveweight gain (g/d) in hoggets fed different levels of ergot alkaloids (µg EV per kg LW)

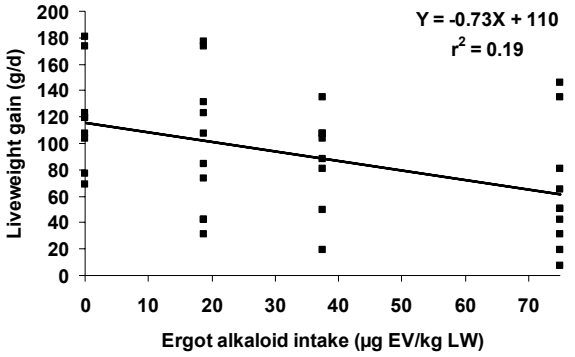
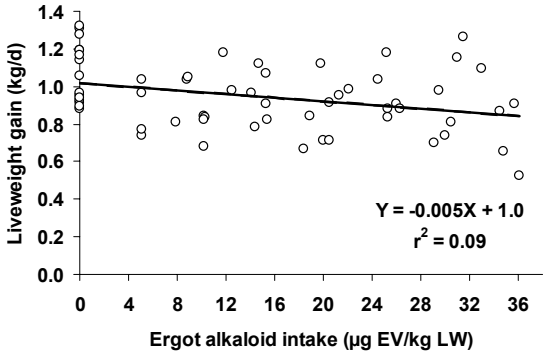


FIGURE 1b: Liveweight gain (kg/d) in heifers fed different levels of ergot alkaloids (µg EV per kg LW).



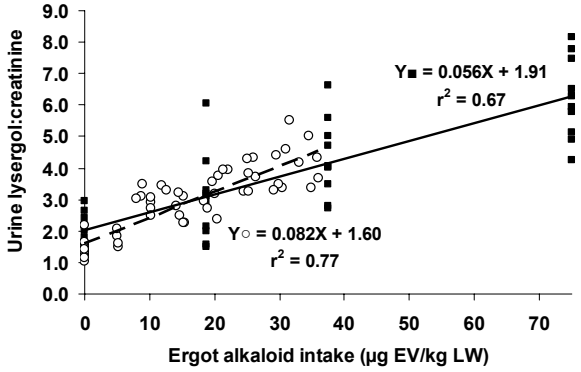
Plasma prolactin

Hogget plasma prolactin concentrations declined markedly in those fed ergot-alkaloids in excess of 18.7 µg EV/kg LW attaining a similar low level for the two higher concentrations (37.5, and 75 µg EV/kg LW). In heifers plasma prolactin concentration averaged over weeks 1, 3 and 6 ranged from 6.2 ± 0.5 ng/ml in control heifers to 3.7 ± 0.5 ng/ml at the highest rates of ergot-alkaloid ingestion (35 µg EV/kg LW) (P<0.01). Plasma prolactin concentration in heifers was reduced by 0.12 ± 0.02 ng/ml for each 1 µg/kg LW EV consumed (P<0.001, r^2=0.30).

Refusals, body temperature and respiration rate

In heifers the average amount of the seed meal refused was higher (P<0.003) at the highest rate of ergot-alkaloid ingestion (28%) compared to controls (2%) and mid-range ergot-alkaloid treatments (1-12%). Average heifer body temperature over weeks 4, 5 and 6 was increased (P<0.01) from 38.9 ^{0}C (0.0 µg EV/kg LW) to 39.2 ^{0}C (35 µg EV/kg LW). There was no effect of treatment on body temperature in hoggets. Under heat challenge, hogget respiration rate was higher (P<0.05) for those receiving the highest levels of ergot alkaloid (75 µg EV/kg LW) compared to controls.

FIGURE 2: Urinary Lysergol:creatinine ratio in hoggets (■ & solid line) and heifers (○ & broken line) fed different ergot alkaloids levels (µg EV per kg LW).



DISCUSSION

As the level of ingested EV increased LWG in hoggets and to a lesser extent in heifers, declined. Over the comparable EV intake range LWG was suppressed by only 15% in heifers but by 27 and 203% in hoggets in experiment 1 and 2 respectively. Comparable overseas studies with sheep report reductions in LWG of 49 and 89% (Gadberry *et al.*, 2003). Fletcher *et al.,* (1999) reported LWG reductions ranging between 17 and 90 % for lambs grazing wild-type vs. nil endophyte ryegrass pastures in Canterbury. In our study the large reduction in hogget LWG in experiment 2 may have been exaggerated by gut fill effects due to the short 14 day monitoring period. The 15% suppression in heifer LWG following high EV intake was less than the 54% reported by Mitzinga *et al.* (1992) but similar to the 13% reported by Emile *et al.,* (2000). Overseas experiments with tall fescue endophyte can be confounded by significant levels of other ergot alkaloids in addition to EV, whereas ryegrass endophyte produces predominantly EV (Gadberry *et al*., 2003).

Hoggets were grazed on relatively poor quality cocksfoot pasture, whereas the heifers grazed high quality chicory which may have prevented a more marked decline in LWG. Stuedemann *et al.* (1998) postulated that with higher quality pasture an increased rate of passage through the rumen may reduce the absorption of ergot-alkaloids and increase the rate of urinary lysergol disappearance.

The clinical symptoms of ergot-alkaloid ingestion are more marked in conditions of high relative humidity and ambient air temperatures (Hannah *et al*., 1990; Spiers *et al.,* 1995; Burke *et al.,* 2001). New Zealand's temperate climatic conditions lessen the impact of ergot-

alkaloids on grazing livestock. As in our study, other grazing experiments in New Zealand (Easton *et al.,* 1999; Thom *et al.,* 1999) have shown only small effects of ergot-alkaloids on cattle productive performance.

In our experiments, the ergot alkaloid treatment diets were ingested in less than 1 hour. In a grazing situation the same intake is spread relatively evenly across the entire grazing period. Our study (results not shown) and other recent studies indicate that when ergot alkaloids (via ground seed) were ingested within 1 hour, urinary lysergyl:creatinine ratio peaked 4-6 hours after feeding (Scannell, 2003). Urine samples were collected 23 hours in hoggets and 6 hours in heifers after the consumption of the rye-seed, but unexpectedly both had comparable urine lysergol ratios for the same EV intake. Scannell (2003) found that 23 hours after treatment, sheep urine lysergol:creatinine ratio were 50% of peak levels. She also showed that peak levels from a single feeding were comparable to the constant level following continuous feeding of ryegrass seed to achieve the same daily EV intake. Clearly there are differences in ergot-alkaloid metabolism in sheep and cattle that require more controlled studies.

In agreement with other studies (Hill *et al*., 2000), our experiment showed that urine lysergol:creatinine ratio was a good indicator of ergot alkaloid ingestion by grazing livestock. Stuedemann *et al.,* (1998) found that 96% of ergot alkaloids were excreted in the urine of steers grazing endophyte-infected tall fescue and these were quantifiable using an ELISA. Over comparable ranges in EV intake from grazed fescue plots, in warm ambient conditions, Hill *et al.,* (2000) reported a reduction of 0.19 kg LW/d ($r^2 = 0.86$) in cattle for each unit increase in lysergol:creatinine ratio, compared to the non significant 0.02 kg LW/d reduction in our study. Our experiment examined only the toxic effects of EV following ingestion and did not include the impact of stock voluntarily reducing intake of high endophyte pastures.

In our study, some of the heifers refused a portion or even all of their daily meal. This effect was more marked as the concentration of ergot-alkaloids in the meal increased. Heifers that repeatedly refused the diets containing higher ergot-alkaloid levels (>20 mg/kg in concentrate meal) were observed to 'smell' their meal before refusing it. The same phenomenon led to the premature cessation of the second hogget experiment.

In conclusion, the lysergol ELISA is an accurate measure of ingested ergot alkaloid intake in both sheep and cattle grazing potentially toxic pastures. In sheep there is a distinct decline in LWG as level of ingested ergot-alkaloid increases but in cattle this trend is much less apparent. The lysergol ELISA test provides a good measure of ergot alkaloid intake but further work is required to relate this to reduced animal performance, particularly in cattle.

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