Development of a feed-intake model for evaluation of toxins associated with endophyte-infected fescue

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Abstract

Feed intake of rats may serve as a sensitive respondent to toxins found in endophyte-infected fescue. Unfortunately, there have been few controlled studies of feed intake to create a reliable model. In the present study, rats were fed diets containing different amounts of endophyte-infected fescue (E+) and ergovaline (EV; a primary toxin associated with fescue toxicosis) to develop a dose-response curve for feed intake. Dose-dependent reduction in feed intake was observed, with a decrease even at the lowest concentration of EV. Involvement of ergotamine (i.e. another toxin in E+) in fescue toxicosis-induced reduction in intake was evaluated by adding it to diets. In contrast to EV treatment, there was only a small reduction in feed intake with ergotamine, followed by rapid recovery to pretreatment levels. A more effective paradigm for evaluating the effect of different toxins on feed intake and potential treatments may be a restricted feed regimen. Rats placed on a 2-h restricted-feed program of ground commercial chow followed by E+ diet show a decrease in both feed intake and growth. Future studies will refine this routine to identify specific effects of toxins found in endophyte-infected fescue on feed intake.

Keywords: rat, fescue toxicosis, feed intake

Introduction

Animals consuming E+ experience decreased blood flow to the extremities, reproductive problems, increased hyperthermia during heat stress, and reductions in both feed intake and body weight gain (Spiers *et al.* 2004). This pathological condition, known as fescue toxicosis, is generally associated with reduced animal health and performance (Oliver 2005). Fescue toxicosis is reported to occur in a variety of animals, including mice

(Varney *et al.* 1991), rats (Spiers *et al.* 2005), sheep (Gadberry *et al.* 2003), and cattle (Oliver 1997). Previous rat and cattle trials, under controlled conditions, have indicated that reduced feed intake is correlated with ergovaline (EV) concentrations in ground seed using a standard diet (Spiers *et al.* 2005; Wax *et al.* 2004). Small amount of EV in ground fescue seed, as determined by HPLC (Rottinghaus *et al.* 1991), decreases feed intake within 24 hours of administration (Eichen *et al.* 2004). However, there has been no systematic determination of the lowest level of EV in the diet that affects feed intake and growth. Likewise, few studies have administered individual ergopeptine alkaloids directly in the diet to determine the effect on a sensitive respondent such as feed intake. Ergotamine was used in the present study to begin this analysis. An inherent problem with any daily measurement, such as feed intake, is determining when the test compound affects the measured outcome. If the response to the compound is delayed, it may be diluted and missed over 24 hours. A restricted-feeding routine, based on a model created by Solis-Salazar *et al.* (2005), is in development to determine the specific effect of selected toxins. By allowing intake only during 2 hours of each 24 hours, we will better characterise the immediate effects associated with toxins found in E+ and eventually evaluate potential treatments that target feed intake. Three rat feeding experiments are described. Experiments 1 and 2 assess rat intake as affected by different levels of EV and ergotamine, respectively, in the feed; Experiment 3 used the restricted-feeding routine to determine the effects of E- tall fescue seed, E+ tall fescue seed, and commercial rat feed diets on feed intake and growth.

Materials and Methods

Experiment 1

Forty-eight adult, male rats (~65 days old) were individually

Figure 1 Feed intake (left) and body weight (right) of rats fed decreasing levels of EV under thermoneutral conditions.

Figure 2 Feed intake (left) and body weight (right) of rats fed ergotamine at different concentrations, under thermoneutral conditions.

housed at 21°C (~50% RH), and maintained on a 12:12 light:dark cycle. Rats were initially fed *ad lib* a diet containing 41.2% ground endophyte-free fescue (E-) seed to establish baseline feed intake. Rats (n=6 per treatment) were then assigned to one of eight treatment diets containing differing amounts of E+ seed to deliver EV at ~2.06, 1.37, 0.687, 0.344, 0.258, 0.179, 0.129, or 0.086 µg EV/g diet. These diets were administered for 8 days, followed by 3 days of E- diet to evaluate recovery of feed intake. Body weight and feed intake were recorded daily at 0900

Experiment 2

A preliminary feeding trial was performed to further examine which ergot alkaloid found in E+ fescue is involved in the reduction of feed intake typically seen in rat studies. Eight adult, male rats (~60 days old) were housed as in the previous experiment. Animals were acclimated to surroundings for 4 days while being fed *ad lib* either a diet containing 41.2% ground E-seed (n=4) or ground chow alone (n=4). Once a baseline feed intake was established (~4 days), ergotamine (EA; 2.06 µg/g

diet) was added to the diets at a concentration similar to that of EV found in E+ seed diets we have fed in previous studies. Ergotamine was mixed with the diet in a commercial mixer for 15 minutes. Rats remained on the diets for 4 days, and then were allowed a 3-day recovery period on diets containing no EA. Following the recovery period, rats were fed diets containing EA at twice the previous concentration (4.12 µg/g diet) for 3 days.

Experiment 3

Six young, adult male rats (~40 days old) were housed, as in the previous experiments, and fed ground commercial diet *ad lib* to establish baseline feed intake level. Animals were placed on a restricted feed diet, with access to ground commercial diet from 1300 – 1500 h. This routine required 11 days to stabilise feed intake, followed by a switch to a diet containing ground E- seed on the restricted feed schedule. After 1 week on the E- diet, rats were switched to E+ diet for 2 weeks. Feed intake and body weight were measured daily at 1500 h.

Figure 3 Feed intake (left) and body weight (right) of rats fed ground commercial diet, E- diet, or E+ diet on a restricted feed schedule, under thermoneutral conditions.

Statistical analysis

For all studies, data were analysed using two-way repeated-measures ANOVA procedure and standard least squares model fit (JMP; SAS Inst., Inc., Cary, NC). Components of the statistical model included treatment, time, and treatment x time interactions. When ANOVA revealed a significant difference in least squares means, a Tukey-Kramer multiple comparisons test (Steel & Torrie, 1980) was performed at $\alpha = 0.05$ to determine both between- and within-treatment effects. All data are reported as least squares means $\pm$ SE.

Results and Discussion

Experiment 1

Feed intake decreased from baseline at all levels of EV, with the greatest decreases in the groups receiving the highest doses (Fig. 1). Rats fed the two highest doses had significant initial decreases in feed intake of 12.7 g and 10.0 g ($\alpha = 0.05$), respectively, while those receiving the lower doses did not significantly decrease feed intake. No treatment group returned to pretreatment feed intake levels by 8 days, but those fed the lowest doses of EV rapidly returned to near-baseline levels. The group fed the highest dose, 2.06 μ g EV/g diet had a lower feed intake during treatment than those fed the five lowest doses: 0.344, 0.258, 0.179, 0.129, 0.086 μ g EV/g diet ($\alpha = 0.05$). Upon return to E- diets, those fed the highest EV concentrations had the largest recovery in feed intake, while those receiving lower doses had smaller increases. Even at very low concentrations of EV in the diet, feed intake in rats was affected. Therefore, determining a threshold level of EV may be quite difficult.

Experiment 2

There was a slight, but not significant ($\alpha > 0.05$), initial decrease in feed intake upon the introduction of EA in the diet in both treatment groups, and there was a return to pretreatment levels after just one day (Fig. 2). Feed intake remained the same during the recovery period when EA was not present in the diet. There was no change in feed intake when the EA level was doubled. Likewise, body weight was not affected by feeding EA. Ergotamine does not appear to be the toxin responsible for feed intake reduction in animals consuming endophyte-infected fescue.

Experiment 3

As expected, feed intake decreased when rats were placed on the restricted feeding schedule. When switched from restricted ground commercial diet to restricted E- diet, feed intake increased slightly, from 13.4 g/d to 16.5 g/d (Fig. 3). Feed intake decreased significantly when rats were switched from the restricted E- diet to the restricted E+ diet, from 16.5 g/d to 11.3 g/d ($\alpha = 0.05$). Growth rate decreased 23.8% when rats were placed on the restricted feed diet, and 94.9% when switched from restricted E- diet to restricted E+ diet. Once on the E+ diet, there was no significant growth ($\alpha > 0.05$).

Implications

Animals consuming E+ diets display a reduction in feed intake, which affects growth and production. By further determining the mechanisms of action of specific ergopeptide alkaloids and the concentrations at which they affect feed intake and feeding

patterns, more effective treatments for fescue toxicosis can be developed.

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