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Use of a Rat Model To Evaluate Tall Fescue Seed Infected with Introduced Strains of *Neotyphodium coenophialum*†

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Experimental cultivars of the pasture grass tall fescue are infected with unique strains of the fungal endophyte *Neotyphodium coenophialum*, which produce low concentrations of ergot alkaloids. A rat model was evaluated as a tool for rapid, initial screening of experimental cultivars considered to be nontoxic. Rats were fed diets that included seed from experimental cultivars of tall fescue with introduced strains of *N. coenophialum* and a toxic control diet containing seed of the cultivar Kentucky 31 (KY31), with its endemic strain of *N. coenophialum*. Rats were preconditioned to a nontoxic diet and then fed treatment diets for 13 days with 5 days at thermoneutrality (21 °C) followed by 8 days under heat stress (31 °C). For most of the 13-day treatment period, rats fed KY31 exhibited depressed daily intake compared to those fed diets of cultivars with introduced endophytes ($P < 0.05$). In addition, rats fed KY31 exhibited significantly less weight than rats on other diets after heat treatment was imposed. For all initial trials and repeated trials, total intake and total gain calculated at the end of each trial were the most consistent indicators of toxicity.

KEYWORDS: Tall fescue; *Festuca arundinacea*; tall fescue toxicosis; endophyte; *Neotyphodium coenophialum*

INTRODUCTION

Tall fescue is one of the most widely grown pasture grasses in the United States (*1*). It can withstand a wide range of biotic and abiotic stresses, partly because it is infected with *Neotyphodium coenophialum* (Morgan-Jones and Gams) Glenn, Bacon, and Hanlin comb. nov., a mutualistic fungal endophyte (*2*). However, tall fescue infected with *N. coenophialum* is also toxic to domestic livestock, causing a severe animal disorder known as "tall fescue toxicosis" (*3*). Tall fescue toxicosis results in part from ergot alkaloids produced by *N. coenophialum* (*4*).

New cultivars of tall fescue are being developed in an effort to balance plant toxicity with plant persistence (*5*). According to this process of development, first proposed for perennial ryegrass (*Lolium perenne* L.) by Latch and Christensen (*6*), tall fescue is treated with heat or fungicides to remove the endemic strain of *N. coenophialum* and then reinfected with unique strains. The unique strains are considered to be almost nontoxic because they produce extremely low concentrations of ergot alkaloids (*4*).

The definitive test for toxicity is a grazing experiment. However, large-scale grazing experiments are expensive and labor-intensive. They require large tracts of land and a multiyear commitment. In addition, they require large quantities of experimental plant cultivars. Ideally, valuable plant germplasm should be screened for toxicity before being consumed in large-scale grazing experiments.

Such a screening procedure may be possible with rat feeding trials. Recent work at the University of Missouri has shown a rat model can mimic certain responses observed in large animal grazing experiments, such as feed intake and body weight (*7*). If the rat model can distinguish toxic from nontoxic germplasm of *Neotyphodium*-infected tall fescue, it can help plant breeders identify which genetic lines to increase for research and commerce and help livestock researchers decide which experimental cultivars to evaluate in large-scale grazing studies.

The objective of this study was to use the rat model to distinguish toxic Kentucky 31 (KY31) from experimental tall fescue cultivars infected with *Neotyphodium* endophytes that are considered to be nontoxic.

MATERIALS AND METHODS

Experiment I: HM4 and HM9. *Diet.* Seed of tall fescue was ground and mixed into a feed ration. In the first experiment, seed included experimental germplasm of "HiMag" tall fescue infected with HM4 and HM9, two strains of *N. coenophialum*. The germplasm with

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HM4 has recently been shown to be nontoxic to cattle (*8*). Seed of KY31 infected with native *N. coenophialum* was also ground and mixed into a ration to serve as the toxic control diet.

Seeds of HM4, HM9, and KY31 were analyzed for ergovaline concentration according to the sample preparation protocol described by Hill et al. (*9*) and the HPLC procedure described by Rottinghuas et al. (*10*). Ergovaline was not detected in the seed of HM4 and HM9 at a detection limit of 50 ppb, whereas KY31 contained 3700 ppb of ergovaline. Additionally, HM4, HM9, and KY31 seeds were also screened for *Claviceps purpurea* ergopeptine alkaloids according to the procedure of Rottinghaus (*11*), and none of these alkaloids were detected at a detection limit of 50 ppb.

The diet contained the following per kilogram of diet: 412 g of tall fescue seed, 23 g of casein, 3.4 g of L-cystine, 40.2 g of mineral mix (AIN 93), 11.5 g of vitamin mix (AIN 93), 100 g of sucrose, 2.9 g of choline bitartrate, and 200 g of corn oil. Water was offered to rats ad libitum throughout the feeding period.

Feeding Period. Rats were Sprague−Dawley strains, males, 60−70 days old. Rats were weighed and then fed a preconditioning diet for 4 days prior to receiving treatment and control diets. The preconditioning diet contained the same ingredients and rations described above, but seed was endophyte-free HiMag. To ensure it was nontoxic, the endophyte-free HiMag was analyzed for ergovaline concentration according to the procedures cited above and found to contain no ergovaline. After preconditioning, rats were fed treatment diets containing seed of HM4, HM9, and the KY31 control. Rats were fed ad libitum for 5 days at thermoneutrality (21 °C). At day 6, the temperature was raised to 31 °C and rats continued receiving diets until day 13. Four rats per treatment were fed individually and served as replications. Body weight and dry matter intake were recorded daily.

Repeated Trial and Data Analysis. The feeding trial was repeated with the same endophytic strains. Different rats were used in the repeat trials. Data from the two trials were combined across feeding trials for statistical analysis. Treatments were arranged in a completely randomized design with four replications per treatment. Treatment means were separated by a least significant difference test and considered to be significant at the 0.05 α level (*12*).

Experiment II: HM3 and HM11. In the second experiment, two additional experimental cultivars were evaluated with the rat model; both lines were considered to be nontoxic. The seed included HiMag tall fescue infected with strains HM3 and HM11 of *N. coenophialum*. As in the first experiment, the control diet in experiment II contained seed from KY31 infected with its endemic endophyte. Diets were formulated, rats fed, and data collected as described for experiment I (above).

Seeds of HM3 and HM11 were analyzed for ergovaline according to the procedures cited above, and ergovaline was not found in either seed at a detection limit of 50 ppb of ergovaline. Seeds of HM3 and HM11 were also analyzed for ergopeptine alkaloids produced by *C. purpurea* as described above; the total ergopeptine alkaloid concentration was 200 ppb for HM3, and none were detected in HM11 at a detection limit of 50 ppb.

The small amount of ergopeptine alkaloids present in HM3, coupled with the presence of visible ergot bodies in the sample, indicates *C. purpurea* infection in the seed sample. Nevertheless, the sample is considered to be nontoxic, especially when compared to the toxic control cultivar, Kentucky 31, which contained an ergovaline concentration of 3700 ppb.

The feeding trial in experiment II was repeated. Data for body weight and dry matter intake were analyzed statistically as described earlier.

RESULTS AND DISCUSSION

Experiment I: HM4 and HM9. *Daily Intake.* Daily intake did not differ across treatments during the preconditioning period (**Figure 1**). The first significant difference occurred immediately after rats were offered treatment diets. On day 1, rats offered the KY31 diet consumed only 64% of the amount consumed by rats fed HM9 and 56% of the amount consumed by rats fed HM4. Over the next 4 days, rats in all treatment groups increased their intake slightly, although rats fed KY31 consistently consumed less than those consuming the HM diets.

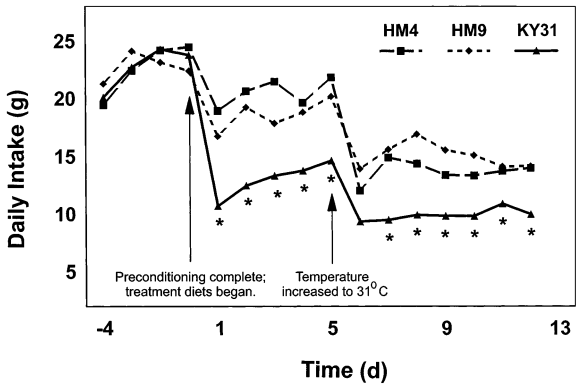

Figure 1. Daily intake of rats fed diets containing seed of three tall fescue germplasms. Germplasms included HiMag infected with introduced strains (HM4 and HM9) of *N. coenophialum* and KY31 infected with an endemic strain. ∗ = significant difference ($P < 0.05$) between germplasms with introduced strains and KY31 with an endemic strain of *N. coenophialum*.

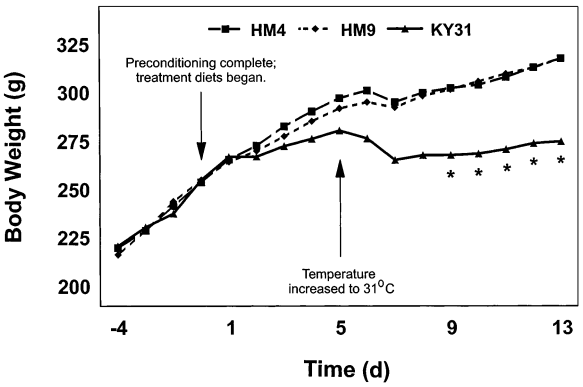

Figure 2. Body weight of rats fed diets containing seed of three tall fescue germplasms. Germplasms included HiMag infected with introduced strains (HM4 and HM9) of *N. coenophialum* and KY31 infected with an endemic native strain. ∗ = significant difference ($P < 0.05$) between germplasms with introduced strains and KY31. with an endemic strain of *N. coenophialum*.

When heat stress was superimposed, rats consuming all diets decreased intake (**Figure 1**). Rats consuming KY31 had already exhibited low intake, so the magnitude of their decrease in intake was not as drastic as that of the other rats. This simultaneous decrease in intake for all rats, coupled with a previously low intake for rats fed KY31, initially prevented a significant difference in daily intake between diet treatments. However, on the day after air temperature was increased, rats consuming diets containing the HM germplasm began a slight recovery, whereas those consuming KY31 diets continued to exhibit low intake. The difference in intake between rats fed the toxic cultivar and those fed the other two cultivars remained significant for the remainder of the experiment.

Body Weight. As with daily intake, body weight of rats did not differ during preconditioning (**Figure 2**). After treatment diets were offered, numerical differences for body weight began to appear. The numerical differences increased throughout the next 5 days, but they were not significant until day 9, 4 days after heat stress was superimposed. Once a significant difference in weight occurred, it remained significant throughout the remainder of the experiment.

Total Intake and Total Gain. As discussed above, the first two figures represent data pooled from two trials within

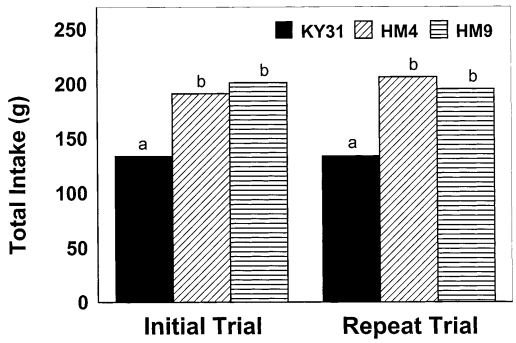

Figure 3. Total intake of rats fed diets containing seed of three tall fescue germplasms, calculated over a 13-day feeding period. Germplasms included HiMag infected with introduced strains (HM4 and HM9) of *N. coenophialum* and KY31 infected with an endemic strain. Rats were fed for 13 days in an initial trial and then a repeat trial. Means with the same letter are not significantly different at 0.05 α level.

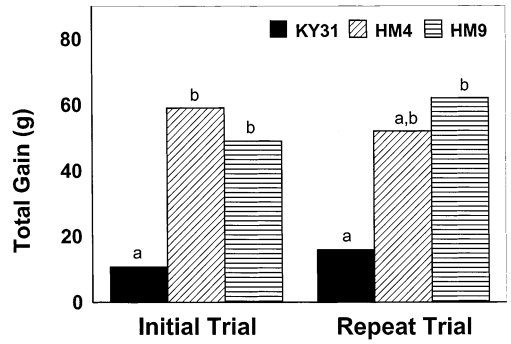

Figure 4. Total gain of rats fed diets containing seed of three tall fescue germplasms, calculated over a 13-day feeding period. Germplasms included HiMag infected with introduced strains (HM4 and HM9) of *N. coenophialum* and KY31 infected with an endemic native strain. Means with the same letter are not significantly different at 0.05 α level. In the repeat trial, gain from HM4 was significantly different from gain from KY31 at 0.07 α level, but the same as HM9.

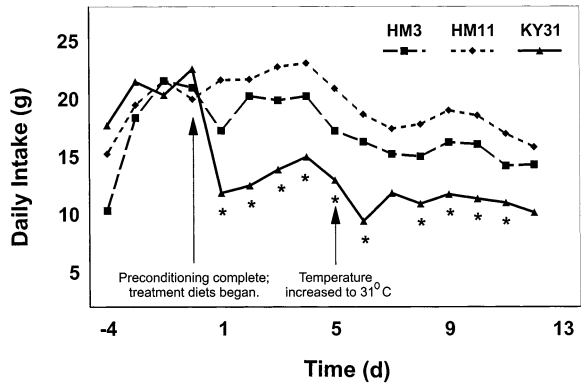

Figure 5. Daily intake of rats fed diets containing seed of three tall fescue germplasms. Germplasms included HiMag infected with introduced strains (HM3 and HM11) of *N. coenophialum* and KY31 infected with an endemic strain. $*$ = significant difference ($P < 0.05$) between germplasms with introduced strains and KY31 with an endemic strain of *N. coenophialum*.

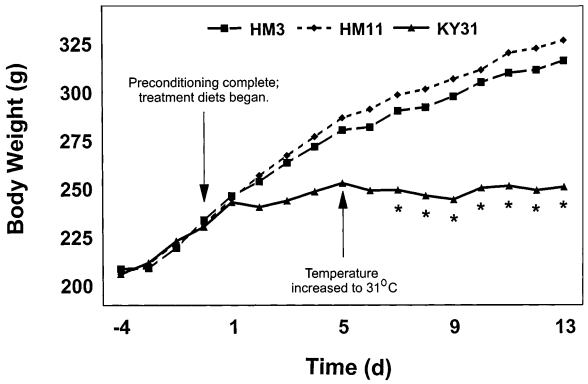

Figure 6. Body weight of rats fed diets containing seed of three tall fescue germplasms. Germplasms included HiMag infected with introduced strains (HM3 and HM11) of *N. coenophialum* and KY31 infected with a native strain. $*$ = significant difference ($P < 0.05$) between germplasms with introduced strains and KY31 with endemic strain of *N. coenophialum*.

experiment I (**Figures 1** and **2**); the pooling was performed to increase power in a means separation analysis. **Figures 3** and **4** illustrate that with some data, pooling was unnecessary to reveal differences. **Figure 3** shows that rats fed KY31 consumed less total diet than those fed HM4 or HM9; this difference occurred in each trial of experiment I, prior to repeating and pooling the data. **Figure 4** shows differences in total gain also differed in individual trials, although the difference in the repeat trial was significant at $\alpha = 0.07$.

The practical importance of this relates to laboratory efficiency, not to statistical tinkering. If only one feeding trial can distinguish toxic germplasm from nontoxic germplasm, only four rats per treatment are required to be fed. This allows plant breeders to surrender only a small portion of valuable experimental seed. Additionally, if the repeat trial is unnecessary, an initial screening can be completed within 17 days. This rapid turnaround time is important to plant breeders in the lower midwestern United States; it would allow them to harvest, clean, and screen their germplasm prior to autumn planting for a new cycle of selection. In Missouri, for example, seed could continue to be harvested in mid-July and cleaned by August 1 and then screened for 17 days prior to planting on September 1.

Experiment II: HM3 and HM11. *Daily Intake.* As in experiment I, there were no significant differences in daily intake during preconditioning (**Figure 5**). Also as in experiment I, feeding the treatment diets caused an immediate decrease in diet intake (KY31). There were two dates when intake did not differ between rats fed the toxic KY31 diet and those fed the HM diets. On day 7, rats fed KY31 increased intake, whereas those fed HM decreased intake. On day 12, these rats did not differ in intake because of variation on that day.

Body Weight. The general trend of body weight that occurred in experiment I repeated in experiment II (**Figure 6**). Rats did not differ in weight until after treatment diets were offered, and, even then, differences were only numerical. The first significant difference occurred on day 7, 2 days after the air temperature was raised to 31 °C. Thereafter, the differences became more pronounced and remained statistically significant.

Total Intake and Total Gain. **Figures 7** and **8** show the same response of experiment I. Rats fed KY31 consumed less total diet and gained less than rats fed HM3 or HM11. Repeating and pooling the data were not necessary to reveal differences between toxic and nontoxic cultivars.

We conclude that a rat feeding trial can provide a rapid tool for screening experimental cultivars of tall fescue for signs of toxicity. The response occurred in each of four independent trials. Rats fed toxic KY31 consumed less and weighed less than rats fed experimental lines considered to be nontoxic. Because of fluctuating data, differences in daily intake were not significant on each day of the trial, even when data were pooled across trials. Differences in total intake and total gain, however, were significant in both the initial and repeat trials,

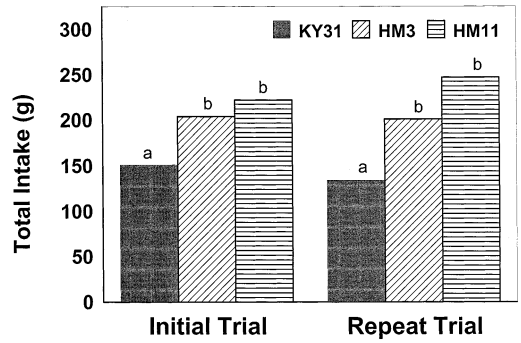

Figure 7. Total intake of rats fed diets containing seed of three tall fescue germplasms, calculated over a 13-day feeding period. Germplasms included HiMag infected with introduced strains (HM3 and HM11) of *N. coenophialum* and KY31 infected with an endemic native strain and fed for 13 days. Means with the same letter are not significantly different at 0.05 α level.

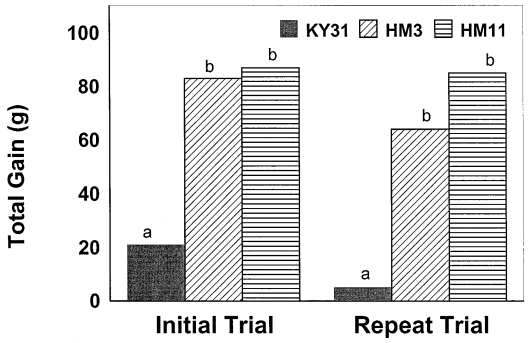

Figure 8. Total gain of rats fed diets containing seed of three tall fescue germplasms, calculated over a 13-day feeding period. Germplasms included HiMag infected with introduced strains (HM3 and HM11) of *N. coenophialum* and KY31 infected with an endemic native strain and fed for 13 days. Means with the same letter are not significantly different at 0.05 α level.

and differences were consistently observed without the need to repeat the trial and pool the data. The repeatability of total intake and total gain suggests that only four rats per treatment and 17 days of feeding are required to distinguish toxic germplasm from that considered to be nontoxic.

The rat feeding trial is not intended to replace livestock grazing trials. Rather, it is intended to provide animal scientists and plant breeders with an early indication of potential toxicosis. This early indication would prevent researchers from inadvertently planting a toxic cultivar in a large-scale grazing experiment. It would also help plant breeders decide which germplasm to increase for further research and marketing.

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