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Effects of Ergot Alkaloids on Food Preference and Satiety in Rabbits, As Assessed with Gene-Knockout Endophytes in Perennial Ryegrass (*Lolium perenne*)

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Neotyphodium species are fungal endophytes best known for their protection of grass hosts and production of bioactive metabolites including ergot alkaloids. Perennial ryegrass−*Neotyphodium* sp. Lp1 symbiota that have altered ergot alkaloid profiles (resulting from knockouts in two different endophyte genes) were fed, along with controls, to rabbits to test the effects of ergot alkaloids on food preference and satiety. Interestingly, rabbits dramatically preferred plants that were endophyte-infected but free of ergot alkaloids over endophyte-free plants ($P = 0.01$). Accumulation of ergot alkaloids of the clavine class counteracted the added appeal of endophyte-infected plants. In satiety tests, consumption of ergovaline (the ultimate ergot pathway product in wild-type endophyte), but not of several other ergot alkaloids, during an initial meal had a negative effect on subsequent rabbit chow consumption ($P < 0.05$). The data indicate that clavines were sufficient to reduce the appeal of endophyte-infected grasses, whereas only ergovaline reduced appetite.

KEYWORDS: Ergot alkaloid; ergovaline; endophyte; feeding deterrent; herbivory; rabbits

INTRODUCTION

Many important forage and turf grasses contain fungi of the genus *Neotyphodium* that grow as endophytic mutualists (*1*, *2*). Without causing visible symptoms, these fungi colonize intercellular spaces of grass tissues and are transmitted vertically via seed. Comparisons of endophyte-infected grasses with endophyte-free varieties have associated endophyte presence in certain grasses with resistance to various stresses including, in some cases, vertebrate herbivory (*3*−*7*). In agricultural settings the antiherbivore effects may be manifested as toxicoses in grazing animals (*8*−*12*).

Some endophytic *Neotyphodium* species produce ergot alkaloids. These ergot alkaloids have been linked to the toxicoses and antimammalian herbivore activities of endophytes in agricultural plants, in particular, in tall fescue infected with its endophyte *Neotyphodium coenophialum* (*9*−*11*, *13*). Many studies have focused on the ergopeptine alkaloid ergovaline, but ergovaline is just one of several ergot alkaloids produced by *N. coenophialum* (*14*, *15*). Ergot alkaloid producing fungi commonly produce complex profiles of ergot alkaloids through accumulation of certain intermediates and diversion of intermediates to shunts off the central ergot alkaloid pathway (*16*). *Neotyphodium* spp. endophytes that produce ergot alkaloids typically accumulate ergovaline as the ultimate product and one or more clavine alkaloids as intermediates or shunt products, as well as simple amides of lysergic acid, such as ergine, as additional shunt products (*14*−*18*) (**Figure 1**).

The ergot alkaloid pathway has been partially characterized in *Neotyphodium* sp. Lp1, a species of perennial ryegrass endophyte separate from the more commonly observed *Neotyphodium lolii* but derived from the hybrid *N. lolii* × *Epichloë typhina* (*19*). *Neotyphodium* sp. Lp1 accumulates high concentrations of ergot alkaloids in a profile similar to that produced by the tall fescue endophyte *N. coenophialum* (*17*) but has a less complex genome and is more amenable to molecular genetic research. Genes encoding two enzymes in the ergot alkaloid pathway of *Neotyphodium* sp. Lp1 have been cloned and characterized. The first pathway-unique step is the prenylation of tryptophan catalyzed by dimethylallyltryptophan (DMATrp) synthase. The gene, *dmaW*, encoding DMATrp synthase has been knocked out by homologous recombination with an altered copy, and perennial ryegrass infected with the *dmaW* knockout mutant contained no ergot alkaloids (*20*). The penultimate step of the pathway is catalyzed by the lysergyl peptide synthetase complex, which is composed of two separate subunits (*21*). The gene *lpsA* encoding lysergyl peptide synthetase 1 (LPS1) was

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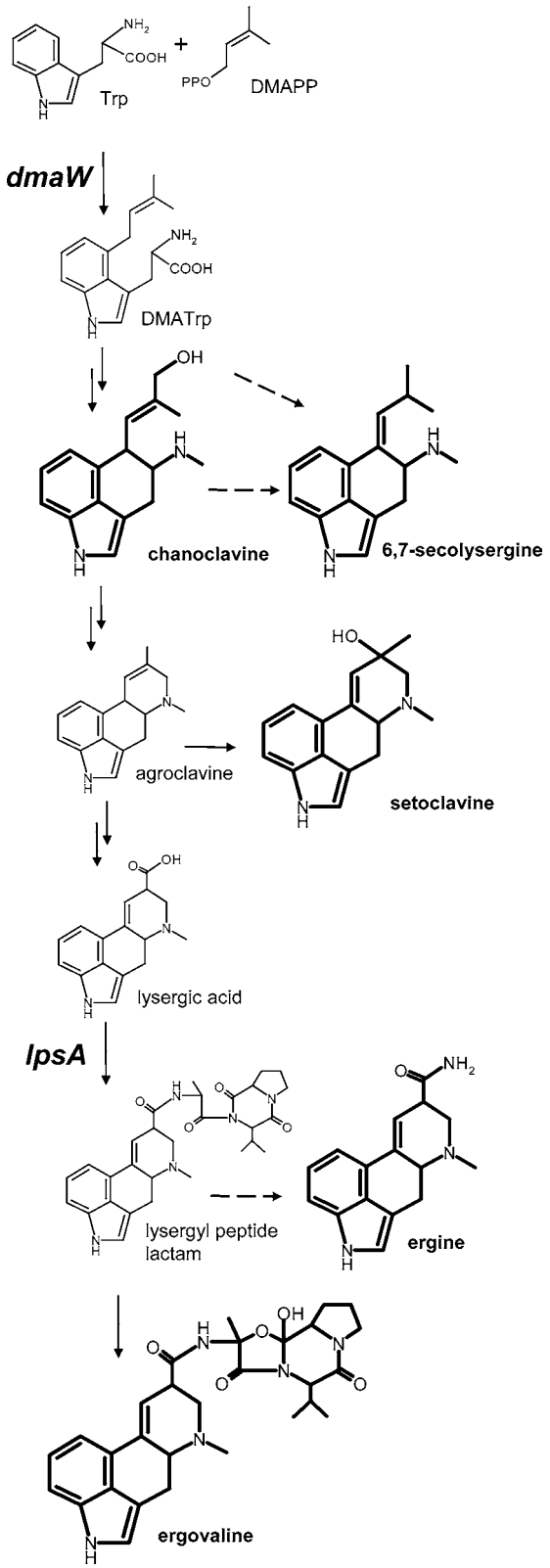

Figure 1. Structures and common names of ergot alkaloids abundant in *Neotyphodium* sp. Lp1–perennial ryegrass associations. Alkaloids most abundant in the leaf tissue of the current study are illustrated in boldface and with larger structures. Additional key intermediates are included to provide context for the abundant alkaloids and for steps inactivated by gene knockout (designated *dmaW* and *lpsA*). Double arrows between alkaloids indicate more than one step between illustrated alkaloids. Dashed arrows represent proposed divergence points of incompletely characterized spurs. DMAPP, dimethylallylpyrophosphate; Trp, tryptophan; DMATrp, dimethylallyltryptophan.

knocked out in *Neotyphodium* sp. Lp1 (*22*). The *lpsA*-knockout strain produced clavine alkaloids and lysergic acid in infected perennial ryegrass but not ergovaline or the lysergic acid amides ergine and lysergyl-alanine, which are typically produced by the wild-type isolate (*17*, *22*).

Perennial ryegrass plants containing the two ergot alkaloid pathway knockout strains provide a means to resolve effects of ergot alkaloids from other endophyte-associated effects, when compared to endophyte-free plants and plants infected with the wild-type endophyte. Because plants containing the modified endophytes were initially available in limited quantities and grown in containment, we sought to investigate the effects of ergot alkaloids on herbivory with relatively small quantities of vegetative material and model animals. Previous studies indicated that rabbits were sensitive to endophyte-associated toxicoses and that endophyte affected food intake in rabbits (*13*, *23*). In this study, we investigated the effects of endophyte-produced ergot alkaloids, as manipulated through the gene knockouts described above, on food preference and satiety in rabbits. The knockout endophytes allowed the separation of ergot alkaloid effects from other potential endophyte effects and also allowed for the separation of classes of ergot alkaloids in otherwise identical endophyte−grass associations.

MATERIALS AND METHODS

Endophytic Fungus−Grass Associations. Four types of endophytic fungus−grass associations (symbiota) differing in ergot alkaloid profiles (**Table 1**) were cultivated in 15-cm diameter pots at the West Virginia University (WVU) Plant and Soil Sciences greenhouses. In each case the host plant was perennial ryegrass (*Lolium perenne*) cv. Rosalin (Advanta Seeds Pacific, Albany, OR). Associations were defined by the following endophyte states: endophyte-free (E-), lacking all ergot alkaloids; wild-type *Neotyphodium* sp. Lp1 endophyte (Wt), containing wild-type ergot alkaloid profile; *lpsA* knockout (*lpsA* ko) of *Neotyphodium* sp. Lp1, producing clavines but not ergovaline or simple amides of lysergic acid (*17*, *22*); and, *dmaW* knockout (*dmaW* ko) of *Neotyphodium* sp. Lp1, producing no ergot alkaloids (*20*). For each of the symbiota, plants derived from six separately infected seedlings were maintained, and material from at least three individuals was pooled in each feeding trial. Pooling of material was intended to minimize any plant genotype effects, because perennial ryegrass is an obligately outcrossing species. Perennial ryegrass samples used in feeding trials were cut at approximately the same time of day (9:00−10:30 a.m.), sealed in plastic bags until the trial began, and offered to rabbits within 30 min of harvest. All experiments involving genetically modified endophytes were conducted in compliance with protocol 2003IBC013 approved by the WVU Institutional Biohazards Committee.

Ergot Alkaloid Analyses. A subsample of every grass−endophyte association used in each feeding trial was analyzed to quantify ergot alkaloids. Alkaloids were extracted as previously described (*17*) with the following modifications. Material analyzed was primarily leaf blade, as opposed to exclusively pseudostem (defined as leaf sheaths of mature leaves, stem, and unfurled leaf blade of the youngest leaf) in the previous study. Material was dried at 50 °C and pulverized in 2-mL, screw-cap microcentrifuge tubes (Fisher Scientific, Pittsburgh, PA) containing 10 3-mm glass beads, along with the extraction solvent, in a FastPrep FP120 (Q-biogene, Irvine,CA) with agitation at 6.5 m/s for 45 s.

Ergot alkaloids were analyzed by HPLC on a 150 mm × 4.6 mm Phenomenex Prodigy 5-μm ODS3 reverse phase C18 column (Phenomenex, Torrance, CA) subjected to a gradient of 5% (v/v) acetonitrile + 95% 50 mM ammonium acetate to 75% acetonitrile + 25% 50 mM ammonium acetate, as described previously (*17*, *24*). Ergot alkaloids other than chanoclavine were detected by fluorescence with excitation at 310 nm and emission at 410 nm and quantified by comparison to an internal standard of ergotamine tartrate (Sigma, St. Louis, MO). Chanoclavine was detected by passing the eluates through a second fluorescence detector with excitation and emission wavelengths of 272

Table 1. Concentrations of Ergot Alkaloids in Leaf Blade Samples Fed to Rabbits in Preference Experiments

endophyte	ergovaline	ergine	6,7-secolysergine	chanoclavine	setoclavine
Wt	1.22 ± 0.14 (2.3)[a]	0.18 ± 0.02 (0.7)	0.49 ± 0.04 (2.0)	1.24 ± 0.16 (4.8)	0.09 ± 0.01 (0.4)
lpsA ko	nd[b]	nd	1.25 ± 0.15 (5.2).	1.18 ± 0.17 (4.6)	0.12 ± 0.01 (0.5)
dmaW ko	nd	nd	nd	nd	nd
E-	nd	nd	nd	nd	nd

[a] The first set of numbers represents the mean for each alkaloid in μg/g of dry weight of plant material ± standard error; in the second set (in parentheses), mass data have been converted to μmol/kg. [b] nd, none detected.

and 372 nm, respectively, and quantified relative to dilutions of agroclavine (Sigma) as an external standard (*25*). Differences in quantities of individual alkaloids accumulating in Wt versus *lpsA* ko symbiota were assessed by Student's *t* test.

Animals. Experiments were conducted with four female New Zealand White rabbits (*Oryctolagus cuniculus*) obtained from Harlan (Indianapolis, IN). Rabbits were 9 weeks old at the onset of the feeding trials, and experiments were conducted periodically over a period of 5 months. Rabbits were housed at the WVU Office of Laboratory Animal Research and were maintained on Purina Mills (St. Louis, MO) high-fiber rabbit chow. Rabbit chow was tested for ergot alkaloids, and none were detected. During the weeks preceding the first feeding trial, rabbits were familiarized with the plant material by feeding them perennial ryegrass infected with wild-type *Neotyphodium* sp. Lp1 as a sole source of food for ≈15 h on four separate occasions. On three other occasions, the rabbits were fed endophyte-free perennial ryegrass under similar conditions. All animal protocols (00-1205) were approved by the WVU Animal Care and Use Committee.

Preference Experiments. Food was withheld for 16 h immediately preceding each trial, and each trial was conducted at approximately the same time of day (9:30−11:00 a.m.). Rabbits were offered equal quantities (5 g) of freshly cut samples of perennial ryegrass defined by two of the four endophyte states described above. Rabbits were allowed up to 20 min to eat, if adequate quantities of both samples remained. If one sample was completely consumed prior to 20 min, the trial was stopped immediately. The amount of time spent eating each of the samples was recorded, and the amount of each grass remaining was measured at the end of the 20 min. All possible pairwise comparisons were made a minimum of nine times and a maximum of 13 times. Each rabbit participated in at least 2 of 9 or 3 of 13 trials with each grass pair combination. Data were analyzed with analysis of variance (ANOVA) techniques, using a nested design within randomized complete blocks. Each rabbit constituted a block. The ANOVA tested for differences among the six possible diet pairs and between diets within each pair. The General Linear Models procedure of SAS (Cary, NC) was used to perform these analyses.

Satiety Experiments. Food was withheld for 16 h immediately preceding each trial, and each trial was conducted at approximately the same time of day (9:30−11:00 a.m.). Rabbits were offered an initial meal of 10 g of one of the four endophyte states of perennial ryegrass and allowed 20 min to eat. After 20 min, there was an 8-min pause in the feeding, during which any remaining grass was weighed. Rabbits were then given free access to 60 g of rabbit chow for 20 min, and the amount of chow consumed in the second course was recorded.

In initial regression analyses, the grams of chow consumed during the second course of the two-course feeding trial was analyzed relative to the following factors: trial date; participating rabbit; endophyte presence or absence (endophyte type was reflected in alkaloid quantities, below); grams of grass eaten in the first course of the trial; and the quantity (in nanomoles) of each individual ergot alkaloid consumed during the first course (measured for each individual sample). This analysis was followed by a backward stepwise selection process until only factors having a significant main effect ($P < 0.05$) remained. Subsequent regression analyses included only three factors—the two significant non-alkaloid factors from the initial analysis (rabbit and grass eaten) and each ergot alkaloid individually. Analyses were conducted with JMP (SAS).

RESULTS AND DISCUSSION

Ergot Alkaloid Content in Leaf Tissue Fed to Rabbits. The mean ergot alkaloid content of samples fed in the preference experiments is shown in **Table 1**, and the positions of these alkaloids in the pathway relative to the two mutated steps are illustrated in **Figure 1**. Endophyte-free plants and plants containing *dmaW* ko contained no ergot alkaloids. Plants infected with Wt endophyte contained a full spectrum of ergot alkaloids including three clavines, the simple amide of lysergic acid (ergine), and ergovaline. Consistent with previously reported results (*17*), the *lpsA* ko produced no detectable ergovaline or ergine but accumulated clavines, including significantly more 6,7-secolysergine (a shunt product from the early stages of the pathway) than did Wt ($P < 0.001$). Differences in concentrations of other clavines were not significant ($P > 0.19$). The shift in alkaloid profiles resulted in the *lpsA* ko accumulating 43% more clavines, on a molar basis, than did Wt, but remarkably the total moles of ergot alkaloid measured in *lpsA* ko as compared to Wt were approximately equal (10.3 μmol/kg of infected plant tissue versus 10.2 μmol/kg, respectively).

In previous studies of the these perennial ryegrass−*Neotyphodium* sp. Lp1 symbiota, two additional ergot alkaloids—lysergic acid and lysergyl-alanine—were measured in plants infected with Wt endophyte, and lysergic acid also was present in plants containing *lpsA* ko endophyte (*17*). In the present study, these alkaloids were only occasionally detected in Wt or *lpsA* ko symbiota and were present in quantities below the limit of accurate quantification, which is ≈40 ng/g of dry weight of plant tissue (*26*). A critical difference between the current analyses and those in the previous paper is the type of plant material analyzed. In the present study, mainly leaf blade material was analyzed because this was the material being harvested and fed to rabbits. In the previous paper, pseudostems exclusively were analyzed because they were hypothesized to be the richest available source of ergot alkaloids. In similar analyses of separated tissues, lysergic acid and lysergyl-alanine also were not detectable, or were below the limit of quantification, in leaf blades of tall fescue−*N. coenophialum* symbiota but were present in quantifiable concentrations in pseudostems of the same plants (unpublished results). Differences in processing of samples—freeze-drying followed by milling in a coffee grinder for the previous analyses (*17*) as compared to air-drying at 50 °C and bead-beating in the current study—also may have contributed to the observed differences in profiles. However, other experiments measuring ergot alkaloids extracted from pseudostem tissue indicated that the two methods produced similar yields (unpublished results). Moreover, the effects of plant tissue on alkaloid yield are well documented (*14*, *26*).

Preference Experiments. Preference tests of *dmaW* ko compared with E- resulted in significantly more *dmaW* ko being eaten than E- ($P = 0.01$; **Figure 2A**). These data indicate that in the case of *Neotyphodium* sp. Lp1, the presence of endophyte in the absence of ergot alkaloids increases the appeal of the

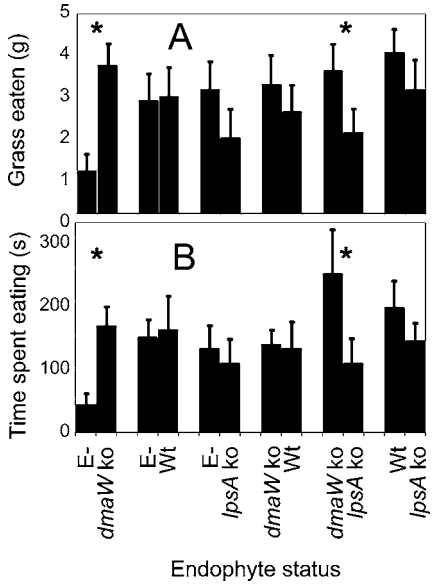

Figure 2. Quantities of (**A**) grass samples consumed and (**B**) time spent eating individual samples by rabbits in preference tests of grasses containing the indicated endophyte or no endophyte. Error bars indicate standard error of the mean. Asterisks indicate a significant difference in means ($P < 0.05$).

infected grass to rabbits. When E- was fed alongside Wt, rabbits demonstrated no preference for either type. Similarly, consumption levels of E- and *lpsA* ko, when offered side-by-side, were not significantly different, although there was a trend toward greater consumption of E-. Because Wt and *lpsA* ko differ from *dmaW* ko only in producing ergot alkaloids, the accumulation of ergot alkaloids in Wt and *lpsA* ko appeared to counteract the appeal of endophyte presence observed in the comparison of *dmaW* ko and E-. The observation that *lpsA* ko was equally, if not more, effective compared with Wt at counteracting the endophyte appeal demonstrates that clavine alkaloids (which are produced in both *lpsA* ko and Wt) are sufficient to offset the endophyte appeal. Ergovaline and lysergic acid amides, which are produced by Wt but eliminated by the *lpsA* ko, are either ineffective in reducing appeal or are redundant with the clavines present.

When *dmaW* ko was fed alongside Wt, more *dmaW* ko was eaten on average, although the difference was not significant (**Figure 2A**). In pairings of the two knockout-containing grasses, significantly more *dmaW* ko was eaten than *lpsA* ko ($P = 0.02$). These data, along with the observation that *lpsA* ko accumulates more clavines than Wt (**Table 1**), are consistent with clavines being important factors in reducing the appeal of *Neotyphodium* sp. Lp1 infected perennial ryegrass. When the two ergot alkaloid producing symbiota (Wt and lpsA ko) were offered together, more Wt was eaten than *lpsA* ko, but the difference was not significant. The same significant differences and trends observed with the mass of grass consumed were reflected in the time spent eating individual grass samples in each of the pairwise comparisons (**Figure 2B**).

In limited previous work on the *Neotyphodium* sp. Lp1−perennial ryegrass association, two mice (in two trials each) showed no preference when offered perennial ryegrass seed infected with Wt (mean mass of seed consumed = 85 ± 15 mg) compared to E- seed of the same variety (mean mass of seed consumed = 72 ± 8 mg) (*27*). This result is consistent with the lack of preference demonstrated by rabbits for Wt versus E- perennial ryegrass observed in our present study.

No previous studies have been published on animal preference and the *Neotyphodium* sp. Lp1−perennial ryegrass association. However, several studies have addressed vertebrate herbivore preference for tall fescue with or without its ergot alkaloid-producing endophyte *N. coenophialum*. Zebra finches (*Taeniopygia guttata*) first preferred endophyte-infected tall fescue seeds to endophyte-free seeds but over several months reversed their preference (*5*). Canada geese (*Branta canadensis*) demonstrated an initial lack of preference, followed by a learned aversion for endophyte-infected grass plots (*6*). In a study of wild birds that presumably had previous experience with endophyte-infected tall fescue, four of five species preferred tall fescue seed that was endophyte-free (*7*).

Similar trends have been observed with mammalian herbivores of tall fescue. Meadow voles (*Microtus pennsylvanicus*) demonstrated a preference for endophyte-free tall fescue in some trials but no preference in others (*4*). Ram lambs and beef heifers preferred endophyte-free tall fescue hay compared to hay from the same cultivar infected with *N. coenophialum* (*8*).

In field plots containing initially equal mixtures of endophyte-infected tall fescue and endophyte-free tall fescue, endophyte infection frequency increased more rapidly in the presence of mammalian herbivores, indicating preferential grazing of endophyte-free tall fescue (*3*). Conversely, in a study of a grass−endophyte association in which no ergot alkaloids are produced (*Festuca arizonica* with its native *Neotyphodium* sp. endophyte), endophyte presence did not deter herbivory (*28*). In fact, at one of six sites in this study, endophyte-infected plants decreased in proportion relative to endophyte-free plants under grazing pressure. Although they represent different grass−endophyte associations, comparison of these two field studies suggests that ergot alkaloids, rather than the fungi themselves or other factors that they may produce, may serve as vertebrate feeding deterrents in *Neotyphodium*-infected grasses.

Because of the separation of ergot alkaloid effects from endophyte effects, the present study provides a more direct demonstration of ergot alkaloids as critical factors in affecting the appeal of a *Neotyphodium* sp. endophyte-infected grass. Our results further indicate that in this particular interaction, the endophyte-infected grass without ergot alkaloids was more appealing than endophyte-free and that the clavine class of ergot alkaloids is sufficient to reduce the appeal.

Satiety Experiments. In initial regression analyses involving all recorded and measured variables, the amount of ergovaline eaten in the first course had a significant negative effect on the amount of rabbit chow eaten in the second course ($P < 0.05$). Two other factors had significant effects on chow consumption ($P < 0.05$). The rabbit that participated in an individual trial was a significant factor, with one rabbit eating less than the other three regardless of treatment. Also, the amount of grass eaten in the first course was positively correlated with the amount of chow eaten in the second course, indicating that on occasions when rabbits ate more grass, they were likely to eat more chow. Trial date, endophyte presence or absence, and the quantities of ergine, setoclavine, chanoclavine, and 6,7-seco-lysergine consumed during the first course did not have a significant effect on chow consumption ($P > 0.24$). After a backward stepwise selection process, a model containing only factors that had a main effect on chow consumption was obtained. In this model, ergovaline eaten in the first course again had a significant negative effect on chow consumption in the second ($P = 0.001$). Rabbit and grass consumption also remained as main effects ($P = 0.001$). After removal of other factors during the backward selection process, ergine and

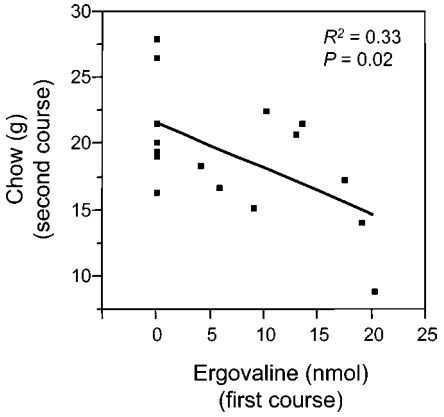

Figure 3. Relationship of ergovaline consumed during the first course of satiety experiments to rabbit chow consumed in the second course. Only data from trials in which rabbits ate at least 9 g of endophyte-infected grass are shown (to minimize confounding effect of grass consumption on chow consumption).

setoclavine appeared to have a positive effect on chow consumption ($P = 0.02$). One possibility is that these particular alkaloids may act as antagonists of ergovaline. However, this proposal was not supported by inclusion of the relevant interaction factors in the model (data not shown). Additional analyses described below did not support ergine and setoclavine as factors affecting chow consumption.

Separate, simplified regression models that included only the two significant non-alkaloid factors from the initial analysis (rabbit and quantity of grass eaten) and an individual ergot alkaloid as factors also indicated that ergovaline consumed in the first course uniquely reduced chow consumption in the second course (model $R^2 = 0.43$; $P = 0.03$ for ergovaline effect). None of the other ergot alkaloids had a significant effect (negative or positive) on chow consumption when substituted for ergovaline in this model (model R^2 values ranged from 0.37 to 0.39; P values for effect of individual ergot alkaloids ranged from 0.23 to 0.66). Potential interactions of main effects, including rabbit with ergovaline, also were investigated, and no interactions were detected.

Direct comparisons of alkaloid factors with chow consumption were complicated by the fact that grass consumption was positively correlated with chow consumption ($R^2 = 0.20$; $P = 0.001$). To provide a visual depiction of the effect of ergovaline on chow consumption, data from trials in which rabbits ate from 9 to 10 g of grass were graphed, minimizing grass consumption as a variable (**Figure 3**). In similar analyses with each of the other ergot alkaloids (including ergine and setoclavine), no significant correlation was found.

The interaction of *Neotyphodium* sp. Lp1 and perennial ryegrass has not been the subject of previously published animal-feeding studies. Most previous work addressing roles for endophytes and ergot alkaloids in limiting food intake has focused on tall fescue with or without *N. coenophialum* in no-choice trials. Durham and Tannenbaum (*29*) found that a diet containing endophyte-infected tall fescue suppressed food and water intake in prairie voles (*Microtus ochrogaster*) relative to a diet containing endophyte-free tall fescue. Similarly, cattle maintained on endophyte-infected tall fescue plots ate less than those kept on endophyte-free plots (*30*). Cattle also ate less endophyte-infected hay than endophyte-free hay or hay containing the ergovaline-deficient endophyte *N. coenophialum* AR542 (*11*). In a study involving rabbits and *N. coenophialum* infected tall fescue, Filipov et al. (*13*) observed that animals maintained on a diet containing endophyte-infected tall fescue consumed less food than those maintained on a diet of endophyte-free tall fescue. Moreover, immunization of rabbits with an antigen consisting of the ergot alkaloid lysergol conjugated to human serum albumin temporarily increased food intake in rabbits consuming *N. coenophialum* infected tall fescue. Antisera collected from similarly immunized animals reacted with several other ergot alkaloids (*10*). Collectively, these data indicate that ergot alkaloids from *N. coenophialum* reduce food intake. Our results extend these observations further to indicate that ergovaline in particular is important for appetite suppression. The association of ergovaline with reduced food intake is consistent with the results of Schöning et al. (*31*), who demonstrated agonist activity of ergovaline at 5-HT_{2A} and 5-$HT_{1B/1D}$ receptors and speculated that this affinity may be responsible for the suppression of appetite observed in cattle grazing endophyte-infected tall fescue.

Implications of Ergot Alkaloid Diversity and Antiherbivore Activities to the Success of Grass−Endophyte Symbioses. Comparisons of plants infected with ergot pathway knockout strains with Wt-infected and E- controls allowed for separation of the effects of endophyte presence from those of ergot alkaloids, as well as for separation of effects of different ergot alkaloid classes. Variation in the concentration of individual alkaloids, which likely resulted from different plant genotypes and multiple harvests over several months, further aided in separating ergot alkaloid effects. The results suggest that different endophyte-produced ergot alkaloids contributed by different means to reducing pressure from mammalian herbivory, thus potentially benefiting the producing fungus and its host grass. The unexpected observation that *Neotyphodium* sp. Lp1 that was modified to not produce any ergot alkaloids (*dmaW* ko) actually enhanced the appeal of perennial ryegrass to rabbits introduces a new facet to the concept of biological protection, suggesting that sometimes alkaloids may be necessary to counteract an otherwise counterprotective effect of an endophyte. Comparisons of pairings involving *lpsA* ko (lacking ergovaline and ergine but containing clavines) or Wt (containing ergovaline, ergine, and clavines) indicated that clavine alkaloids were sufficient to reduce the appeal of the endophyte-infected grass and that ergovaline and ergine were either insignificant or redundant with clavine alkaloids in affecting food preference in rabbits. Conversely, data from the satiety experiments indicated that ergovaline, but not clavines or other measured ergot alkaloids, suppressed appetite. Together these observations support the hypothesis that the accumulation of different ergot alkaloids, as opposed to a singular pathway product, can benefit the producing fungus (*16*).

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