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# Impact of consuming tall fescue seeds infected with the endophytic fungus, *Neotyphodium coenophialum*, on reproduction of chickens

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## Abstract

Most of the 14 million hectares of pastures of tall fescue (*Festuca arundinacea*) planted in the United States are infected with the endophytic fungus, *Neotyphodium coenophialum*. I examined whether the consumption of fescue seed infected with this fungus had an adverse impact on reproduction in birds. Chickens were used as the model; they were maintained on a diet of chick starter mixed in equal amounts with either infected fescue seed (fungus-fed chickens) or uninfected fescue seed (control chickens). Consumption of infected fescue seed slowed the growth rates of female chicks but not males. Fungus-fed hens gained weight more slowly than control hens and also produced fewer eggs. Egg weight and fertility were similar in both groups of hens. Fungus-fed hens were more likely than control hens to produce eggs which lacked a shell. Naive hens exhibited an aversion for infected fescue seed when the seed was first presented to them, but the aversion disappeared after the hens were placed on a diet which included fescue seed for 3 or 6 months. Hens did not discriminate between feed containing infected seed and feed containing uninfected seed.

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## 1. Introduction

In the 1940s, agricultural scientists identified a cultivar of tall fescue (*Festuca arundinacea*) which was superior to other cultivars in its hardiness and productivity; it was named Kentucky 31 or K-31 [1–3]. By the 1970s, this had become the most popular cultivar of tall

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fescue in the United States [1,4]. Unknown at the time, this cultivar was infected with the endophytic fungus, *Neotyphodium coenophialum*, which grows within the plants' leaves, stems, and seeds. This fungus does not undergo sexual reproduction or sporulation and cannot spread to uninfected plants; instead, it is seedborne [5,6]. It also produces ergopeptine alkaloids [7,8] and makes infected grass less palatable and more toxic to herbivorous insects and nematodes [7,9–11]. Hence the relationship between grass and fungus is mutualistic.

Livestock on a diet of fungus-infected tall fescue (hereafter called infected fescue) become ill and can experience lower weight gains, higher body temperatures, reduced rates of conception, and suppressed milk production [12]. Such problems, however, usually can be avoided if livestock are allowed to eat only limited amounts of infected fescue [13].

While farmers and ranchers can limit access of livestock to infected pastures, it is not as easy to do this with free-ranging wildlife. Consumption of infected fescue may be having a largely unrecognized, but adverse, impact on wildlife populations. For instance, consumption of infected fescue seed or leaves can reduce reproductive rates and increase mortality rates of meadow voles (*Microtus pennsylvanicus*) [14], prairie voles (*Microtus ochrogaster*) [15,16], and white-footed mice (*Peromyscus leucopus*) [17].

Many free-ranging birds, such as Canada geese (*Branta canadensis*), feed in fescue pastures where they consume infected fescue plants or seeds. Captive Canada geese fed infected fescue lost weight while those fed uninfected fescue gained weight [18]. Granivorous birds which consume infected seed may also be at risk. Zavos et al. [19] reported a 10% reduction in fertility of Japanese quail (*Coturnix japonica*) when on a diet of infected fescue seeds. Captive zebra finches (*Taeniopygia guttata*) maintained on a diet of infected fescue seed did not differ in weight or reproductive rates from conspecifics maintained on a diet of uninfected fescue seed when ambient temperatures were between 21 and 23 °C, but finches on a diet containing infected seed suffered a higher mortality rate when ambient temperatures were 31–34 °C [20]. In this study, I examined whether consuming tall fescue seeds infected with *Neotyphodium coenophialus* reduces the reproductive ability of chickens.

## 2. Materials and methods

Experiments were conducted at the Utah State University's Poultry Farm between 1994 and 1996. The subjects were White Leghorn chickens obtained as newly hatched chicks from a local poultry breeder. At 3 weeks of age, all chickens were given a uniquely numbered leg band so that they could be individually recognized and were randomly assigned to one of two groups. One group (hereafter referred to as the fungus-fed chickens or fungus-fed hens) was maintained on a feed containing tall fescue seeds (K-31) infected with the fungus *N. coenophialum*. This feed was produced by first grinding infected fescue seeds into a powder using a Wiley Mill. This powder was then mixed with an equal weight of Intermountain West<sup>®</sup> Chick Starter (Smithfield, UT, USA) so that the chickens could not eat the chicken feed without eating the ground fescue seed. The other group of chickens (control chickens) was given an identical feed

except that theirs was made with seeds from uninfected K-31 plants. All chickens were given their feed, a source of calcium (crushed oyster shells), and water ad libitum throughout the experiments.

Fungus-fed chickens were housed in groups inside two of eight floor cages (each 3 m × 4 m × 4 m) while the control chickens were housed in two of the other cages. These groups were switched among the eight floor cages. Groups contained both sexes.

### 2.1. Chick growth rates

To assess whether the consumption of infected fescue seeds reduced the growth rate of chicks, I randomly selected 40 fungus-fed chicks (23 males and 17 females) and 41 control chicks (24 males and 17 females). Each chick was weighed every 2 weeks starting when it was 3 weeks old and ending when it was 11 weeks old. I compared the weight data of fungus-fed and control chicks using a repeated measures analysis of variance. Males and females were analyzed separately. Results of this and all other statistical tests were considered significant if  $P < 0.05$ .

### 2.2. Reproduction

Once the birds were 6 months old and had started laying, 20 hens from the fungus-fed group and 20 hens from the control group were randomly selected and housed individually in a brooding battery which consisted of a series of individual cages, each 0.5 m × 0.7 m × 0.7 m for 4 days per week. During the other 3 days each week, these hens were returned to the large cages containing the other fungus-fed or control birds. Each hen was weighed monthly. While in their individual laying cages, eggs from each hen were collected, counted, and weighed. Each week, I calculated for each hen its egg production rate and the mean weight of its eggs. These data were analyzed using a repeated measures analysis of variance.

The fertility of eggs produced by each fungus-fed and control hen was assessed separately by placing individually marked eggs in a Petersine<sup>®</sup> incubator (Gettsyburg, OH, USA) so that I could identify which hen laid each egg. A total of 157 eggs from fungus-fed hens and 169 eggs from control hens were left in the incubator until the embryos could be identified. I then determined a separate fertility rate for each hen based on the proportion of her eggs that were fertile. Hence, each hen was the experimental unit and not each egg. Fertility rates of fungus-fed hens were compared to control hens using an unpaired *t*-test.

To determine the hatching rate of eggs laid by each fungus-fed and control hen, 413 eggs produced by fungus hens and 338 eggs produced by control hens were left in the incubator long enough to hatch. For each hen, I determined the proportion of its eggs that hatched, and compared fungus-fed to control hens using an unpaired *t*-test.

### 2.3. Food preference tests

I conducted a feeding test every 3 months to assess if hens preferred uninfected or infected seeds. For this test, each individually caged hen used in the reproduction

experiment was allowed access for 24 h to two bowls: one containing infected seeds and the other uninfected seeds. The amount consumed was corrected for water loss by simultaneously placing other bowls of seed in unoccupied cages. The weight loss of seeds in these bowls was then subtracted from the weight loss of feed in bowls in occupied cages. A food preference test was also conducted using infected and uninfected feed. This test was identical to the food preference test using seeds except that this time each hen was given a bowl of infected feed (the same feed on which the fungus-fed chickens were maintained) and another bowl of uninfected feed (the same feed on which control chickens were maintained).

### 3. Results

#### 3.1. Chick growth rates

Growth rates of male chicks were similar ( $F = 0.32$ ; d.f. = 1, 45;  $P = 0.57$ ) between those fed a diet containing infected seed ( $n = 23$ ) and those fed uninfected seed

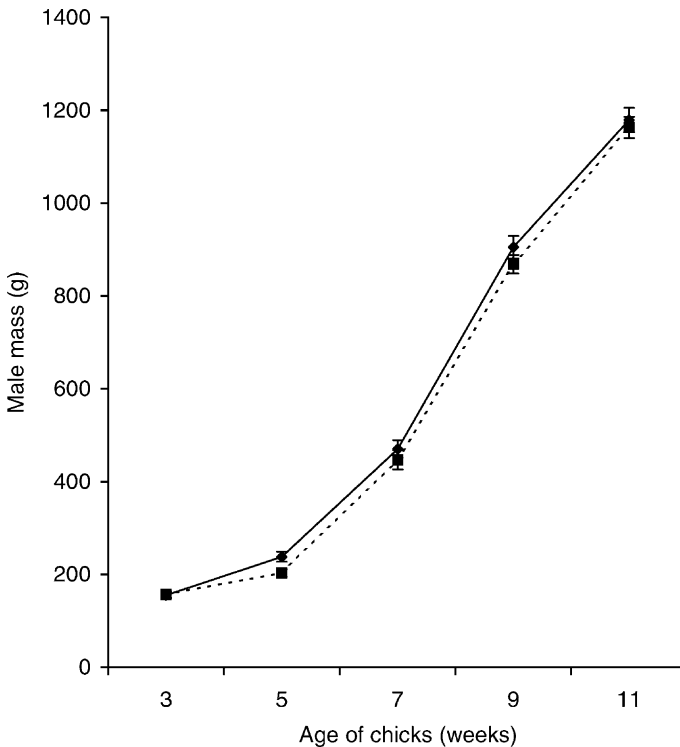


Fig. 1. Mean weight of male chicks maintained on a diet containing tall fescue seeds infected with the endophytic fungus *N. coenophialum* (- - ■ - -) and other chicks maintained on a diet contained uninfected tall fescue seeds (—◆—).

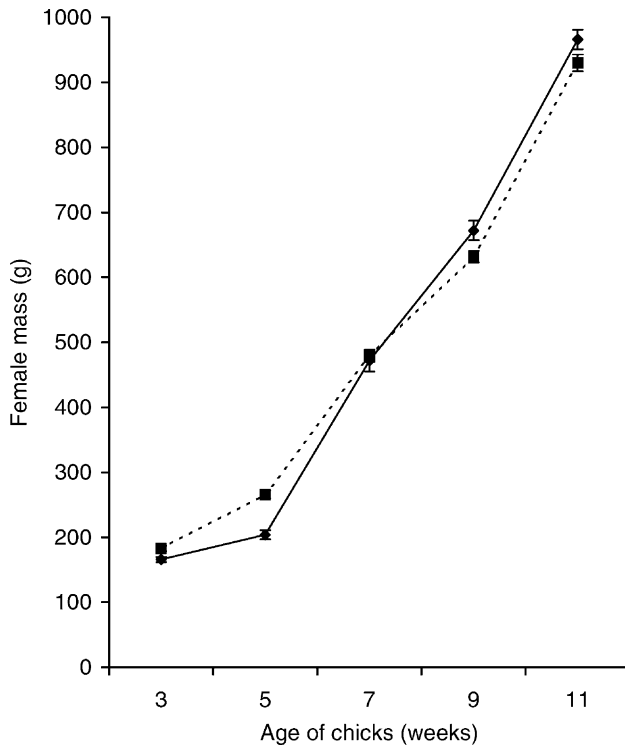


Fig. 2. Mean weight of female chicks maintained on a diet containing tall fescue seeds infected with the endophytic fungus *N. coenophialum* (- - ■ - -) and other chicks maintained on a diet contained uninfected tall fescue seeds (—◆—).

( $n = 24$ , Fig. 1). Weight of female chicks fed a fungus-infected diet ( $n = 17$ ) and those fed an uninfected diet ( $n = 17$ ) also did not significantly differ ( $F = 0.72$ ; d.f. = 1, 32;  $P = 0.72$ ) during the period. Nonetheless, there was a significant interaction for females between treatment and date ( $F = 6.07$ ; d.f. = 4, 128;  $P = 0.0002$ ) which resulted because fungus-fed females were heavier initially than control females, but grew more slowly, so that by the end of the 2-month period, fungus-fed females weighed less than control females (Fig. 2).

### 3.2. Growth rates and egg production of hens

Weight of adult hens varied among months ( $F = 19.95$ ; d.f. = 5, 105;  $P = 0.0001$ ) with weight increasing during the first 4 months of the experiment and then decreasing during the last 2 months (Fig. 3). Throughout this 6-month period, fungus-fed hens were significantly lighter than control hens ( $F = 5.82$ ; d.f. = 1, 21;  $P = 0.025$ ).

Egg production varied significantly by date ( $F = 5.54$ ; d.f. = 5, 110;  $P = 0.001$ ) and treatment group ( $F = 11.74$ ; d.f. = 1, 22;  $P = 0.002$ ) with egg production by fungus-fed hens ( $\bar{x} = 0.60$  eggs/day; S.E.M. = 0.02) being lower than that of control hens ( $\bar{x} = 0.70$ ;

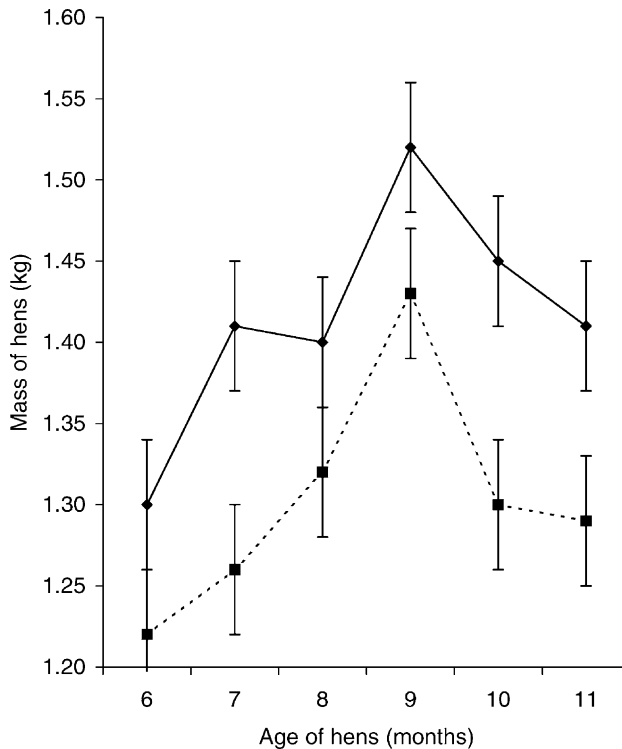


Fig. 3. Mean weight of adult hens maintained on a diet containing tall fescue seeds infected with the endophytic fungus *N. coenophialum* (- - ■ - -) and of hens maintained on a diet contained uninfected tall fescue seeds (—◆—).

S.E.M. = 0.02) throughout the 6-month period (Fig. 4). Mean egg weight increased significantly ( $F = 36.55$ ; d.f. = 5, 105;  $P < 0.0001$ ) in both fungus-fed and control hens during the 6-month period (Fig. 5) but there was no significant difference between fungus-fed and control hens in the weight of their eggs ( $F = 0.01$ ; d.f. = 1, 21;  $P = 0.94$ ).

There was no difference ( $t = 1.11$ ;  $P = 0.27$ ) in the fertility rate of eggs produced by fungus-fed hens ( $\bar{x} = 96.4\%$ ; S.E.M. = 1.0) and control hens ( $\bar{x} = 92.3\%$ ; S.E.M. = 3.4). There also were no differences in the percentage of eggs which hatched between fungus-fed hens ( $\bar{x} = 68.1\%$ ; S.E.M. = 4.8) and control hens ( $\bar{x} = 70.2\%$ ; S.E.M. = 6.9). One interesting difference, however, was that 21 eggs produced by 11 different fungus-fed hens lacked a calcium shell while control hens produced only one such egg. This difference was statistically significant.

### 3.3. Mortality rates

No fungus-fed or control chicks died during the time they were on the fescue seed diet (3–12 weeks of age). During the first week after hens were placed in individual brooder cages, one control hen and five fungus hens died, apparently because they were not familiar

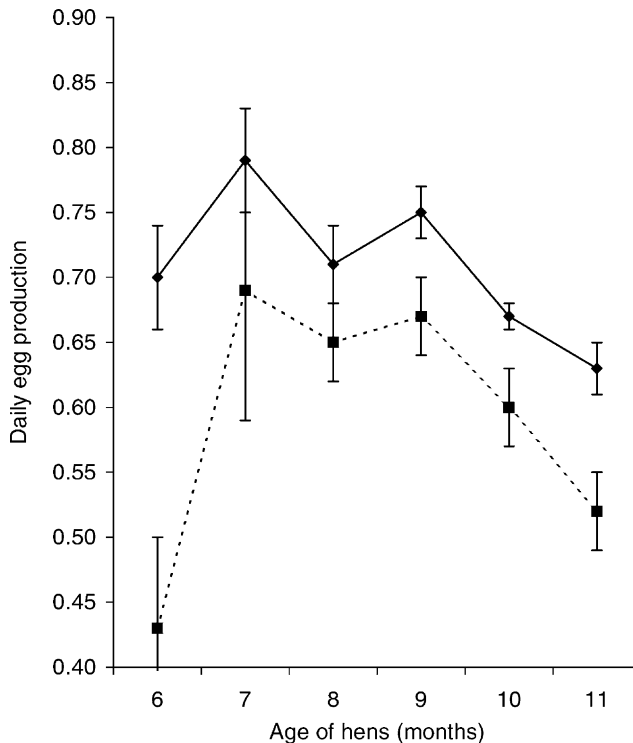


Fig. 4. Mean number of eggs produced daily by hens maintained on a diet containing tall fescue seeds infected with the endophytic fungus *N. coenophialum* (- - ■ - -) and by hens maintained on a diet contained uninfected tall fescue seeds (—◆—).

with the new automatic watering devices located in each brooder cage. These birds were replaced by other fungus-fed and control birds of the same age before I started collecting data on egg production. After the birds had been in the brooder cages for a week and become familiar with them, only one fungus-fed hen and one control hen died during the rest of the experiments.

### 3.4. Food preference tests

Naive chickens tasting tall fescue seed for the first time exhibited a preference for uninfected seed over infected seed when given simultaneous access to both types of seeds (Table 1). However, when these seeds were ground up and mixed in with chicken feed, naive chickens did not discriminate between them (Table 2).

After being on a fescue seed diet for 3 months, both fungus-fed hens ( $t = 0.23$ ; d.f. = 20;  $P = 0.82$ ) and control hens ( $t = 1.07$ ; d.f. = 22;  $P = 0.31$ ) failed to discriminate between infected seed and uninfected seed (Table 1). Likewise, neither fungus-fed hens ( $t = 0.09$ ; d.f. = 22;  $P = 0.93$ ) nor control hens ( $t = 1.00$ ; d.f. = 20;  $P = 0.34$ ) distinguished between chicken feed containing infected seed or uninfected seed (Table 2).

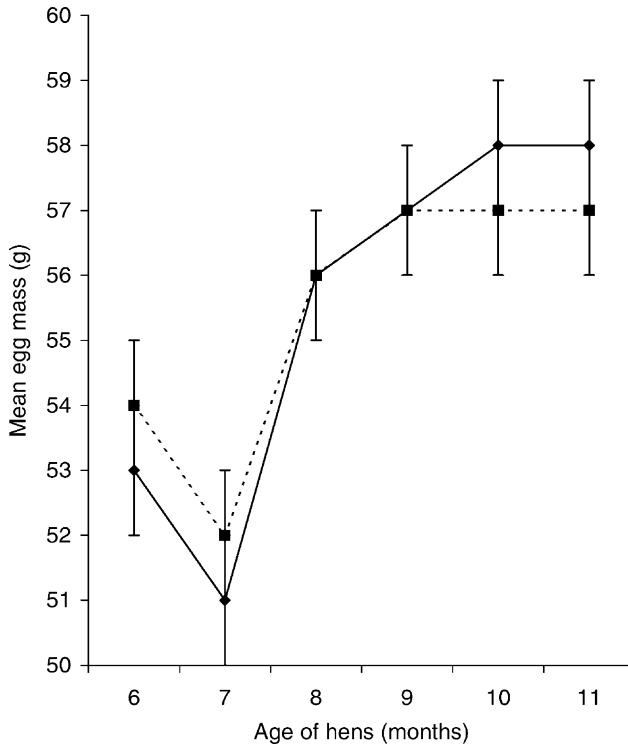


Fig. 5. Mean weight of eggs produced by hens maintained on a diet containing tall fescue seeds infected with the endophytic fungus *N. coenophialum* (- - ■ - -) and by hens maintained on a diet containing uninfected tall fescue seeds (—◆—).

After being on a fescue seed diet for 6 months, both fungus-fed hens ( $t = 0.17$ ; d.f. = 28;  $P = 0.87$ ) and control hens ( $t = 2.16$ ; d.f. = 20;  $P = 0.06$ ) failed to discriminate between infected seed and uninfected seed. In general, both groups of hens ate very little whole fescue seed during these feeding trials (Table 1). These hens continued to consume equal amounts of feed mixed with uninfected seed and feed mixed with infected seed (Table 2).

Table 1

Results of feeding trials in which hens were given access simultaneously to two feeding trays: one containing tall fescue seeds infected with *N. coenophialum* and the other containing tall fescue seeds that were uninfected

Months from start of a fescue seed diet	Grams consumed/day ( $\bar{x} \pm$ S.E.M.)		d.f.	$t$	$P$ (two-tailed)
	Uninfected seeds	Infected seeds			
0	2.9 $\pm$ 0.9	0.3 $\pm$ 0.1	52	2.77	0.01
3	11.8 $\pm$ 2.5	10.1 $\pm$ 2.4	44	0.60	0.56
6	5.1 $\pm$ 1.8	6.3 $\pm$ 1.6	52	0.63	0.54

Table 2

Results of feeding trials in which hens were given access simultaneously to two feeding trays: one containing chicken feed mixed with tall fescue seeds infected with *N. coenophialum* and the other containing chicken feed mixed with tall fescue seeds that were uninfected

Months from start of a fescue diet	Grams consumed/day ( $\bar{x} \pm$ S.E.M.)		d.f.	<i>t</i>	<i>P</i> (two-tailed)
	Uninfected seeds	Infected seeds			
0	13.8 $\pm$ 3.0	17.1 $\pm$ 2.9	50	0.73	0.47
3	75.9 $\pm$ 2.7	73.5 $\pm$ 3.9	44	0.63	0.33
6	66.6 $\pm$ 3.4	58.2 $\pm$ 4.3	46	2.06	0.06

#### 4. Discussion

When fed seeds or leaves from tall fescue plants infected with *N. coenophialum*, both large and small mammals experience a decrease in reproductive rates because of problems such as delayed estrus, lower pregnancy rates, increased abortion rates, smaller litters, and poor lactation in females and reduced fertility in males [21–26]. Whether consuming infected fescue leaves or seeds would adversely impact reproduction in birds is less clear. Zavos et al. [19] reported a reduction in fertility of Japanese quail maintained on a diet of infected fescue seeds. Conover and Messmer [18] found no difference in the reproductive ability of captive zebra finches maintained on a diet of infected fescue seeds and other conspecifics maintained on a diet of uninfected fescue seeds when ambient temperatures were maintained at 21–23 °C.

In this study, I found that consumption by hens of seeds from infected tall fescue had no effect on the weight, fertility, or hatching rates of their eggs. However, fungus-fed hens produced eggs at a slower rate than control hens. This may have been a result of fungus-fed hens being in a poorer nutritional state than control hens and thus unable to sustain the high energy demands of rapid egg laying. Support for this hypothesis comes from the finding that fungus-fed hens were unable to sustain the same body weight as control hens during the egg laying period. Likewise, Canada geese lost weight when they grazed in infected fescue pastures but gained weight when in uninfected fescue pastures [18].

In addition to causing adult hens to have a lower weight than control hens, consumption of infected seed caused female chicks to grow more slowly than control chicks. This effect was sex specific because the growth rates of male chicks did not vary by treatment. Among free-ranging birds, a slower growth rate could cause a significant increase in the mortality rate among the young, and lower their recruitment rate into the breeding population.

Naive chickens preferred uninfected seed over infected ones when given a choice, but this preference disappeared as chickens gained more experience consuming tall fescue seeds, perhaps because experienced hens reduced their consumption of both infected and uninfected fescue seed. The opposite pattern was observed in Canada geese [18] and zebra finches [20]. In these species, discrimination between infected and uninfected tall fescue was exhibited by experienced birds, but not naive ones.

Despite the ill effects that livestock suffer when grazing fungus-infected fescue, farmers still like to plant it in pastures because infected grass is hardier, more resistant to insects,

and outproduces uninfected pastures. Because of these beneficial qualities, infected fescue is also used increasingly for lawns [27]. Fortunately, problems that livestock face when grazing infected fescue can be reduced by limiting the intake of infected fescue. Unfortunately, controlling the forage intake of free-ranging birds is not as easy. My results indicate that consumption of infected fescue seeds may impact egg production of adult chickens and the growth rate of young females. Considering that there are 14 million hectares of tall fescue pastures in the United States and most are infected [1,4], it is important to determine the impact these grasses may be having on free-ranging birds which consume their seeds.

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