Reproductive responses of ram lambs under short-term exposure to endophyte-infected tall fescue seed

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Abstract

Our objective was to determine the influence of short-term exposure to endophyte-infected tall fescue on reproductive function of ram lambs. Rams (214 days of age) were fed a diet free of endophyte-infected fescue seed (EF; n = 8) or endophyte-infected fescue seed (EI; n = 9; 34% of diet; 4.8 μg g⁻¹ ergovaline) for 6 weeks. Feed offered to EF rams, individually fed, was reduced to the average intake of EI lambs from previous day so that intake was similar between treatments and averaged 2.4% BW (DM basis), leading to daily intake of 33.7 μg ergovaline kg⁻¹ BW for the EI fed lambs. Daily high ambient temperature for the trial ranged between 16 and 26 °C. Respiration rate and rectal temperature were measured at 14:00 daily. Blood was collected for serum concentrations of prolactin (weekly) and testosterone (twice weekly). Body weight and body condition scores (BCS; 1 = thin; 5 = fat) were determined every 14 days. Scrotal circumference, scrotal skin temperature, and semen characteristics were determined weekly. Rams were slaughtered after 6 weeks of feeding. Signs of fescue toxicosis in EI fed rams included increased rectal temperature (P < 0.001, R² = 0.11) and respiration rate (day, P < 0.001, R² = 0.25) when high ambient temperature exceeded 22 °C and reduced serum concentrations of prolactin (diet × day, P < 0.001). Body weight of EI fed rams tended to decrease after 36 days of feeding compared with EF fed rams (−3.0 kg versus 0.51 kg; P < 0.07) and BCS was similar between treatment throughout the trial. Serum concentrations of testosterone were greater in EI compared with EF fed rams (diet × day, P < 0.005, R² = 0.08). Scrotal skin temperature, scrotal circumference, semen volume, percent sperm motility, and percent abnormal sperm were similar between treatments. Spermatozoa concentration tended to be greater in EF compared with EI fed rams after 43 days of feeding (P < 0.10; R² = 0.15). Rate of forward movement of spermatozoa tended to increase at a greater rate between Days 8 and 29 in EF compared with EI fed rams (diet × day, P < 0.08). Feeding endophyte-infected fescue seed to ram lambs was associated with potential decreased fertility and increased serum concentrations of testosterone. Short term exposure of endophyte toxins to male ruminants may negatively impact reproductive responses. Feeding for longer periods may further reduce fertility and merits further research.

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1. Introduction

Tall fescue (*Festuca arundinacea*), a cool season grass grown on more than 14.2 million ha in the US, can be associated with fescue toxicosis in grazing animals (Ball et al., 1996). Reproductive losses in cattle associated with fescue toxicosis exceed $300 million annually (Hoveland, 1993). An endophytic fungus (*Neotyphodium coenophialum*), that grows within the plant, enables the plant to be more tolerant of drought, nematodes, and insects. However, the host–endophyte association produces a range of alkaloids, including ergot alkaloids, that when consumed, often reduces weight gains, conception and pregnancy rates. Signs of fescue toxicosis include elevated core body temperature and respiration rate, especially during heat stress. Circulating prolactin is often decreased because the ergot alkaloids act as dopaminergic agonists (Muller-Schweinitzer et al., 1978).

Poor reproductive performance in cattle, or decreased pregnancy and calving rates (Brown et al., 1992, 1997, 2000; Gay et al., 1988), has been attributed, in part, to changes in follicular and luteal dynamics (Mahmood et al., 1994; Burke et al., 2001) and delayed conception (Bond et al., 1988). It is not understood how fescue toxins affect fertility of male ruminants. Breeding bulls and rams are often maintained on pastures free of tall fescue. However, during breeding, males may be moved to tall fescue pastures with ewes or cows. Decreased circulating prolactin and increased rectal temperature have been used as indicators of fescue toxicosis and will often change within a few days exposure to endophyte-infected tall fescue, especially during heat stress (Burke et al., 2001; Burke, unpublished data in ewes). Bromocryptine-induced hypoprolactinemia in rams reduced testosterone, semen volume, scrotal circumference (Regisford and Katz, 1993), or delayed testicular development (Barenton and Pelletier, 1980). Testicular development and sperm production was reduced in male rats fed endophyte-infected fescue seed (Zavos et al., 1986).

The aim of this study was to quantify testicular development by evaluating sperm production, scrotal circumference and serum concentrations of testosterone in peri-pubertal rams fed an endophyte-free or infected fescue seed diet for 43 days, typical of ergot alkaloid exposure during the breeding season. The use of a seed diet allows feeding of a higher concentration of ergot alkaloids and quantification of ergovaline, an ergot alkaloid associated with fescue toxicosis. Concentrations of ergovaline in tall fescue seed and forage ranges from 0.1 to 6.0 µg g⁻¹ and fluctuates with season (Belesky et al., 1988; Hill et al., 1991; Porter, 1995) and may be too low in hay to induce fescue toxicosis (Emile et al., 2000).

2. Materials and methods

All experimental procedures were reviewed and approved by the USDA, Agricultural Research Service Animal Care and Use Committee in accordance to National Institute of Health guidelines for Care and Use of Laboratory Animals. Pain and stress to sheep were minimized throughout the experiment.

Seventeen Hampshire (n = 9) or Suffolk (n = 8) ram lambs approximately 214 days of age and weighing 58.6 ± 3.7 kg, were blocked by breed and randomly assigned to a diet free of endophyte-infected fescue seed (EF; n = 8) or infected tall fescue seed (EI; n = 9) in October 2002 for a 6-week period. Before the experiment began, ram lambs were group fed to meet NRC (1985) requirements. The EF diet consisted of 50% oats, 40% chopped bermudagrass hay, 4% soybean meal, 5% molasses, 0.5% limestone, 0.5% ammonium chloride, and 0.15% vitamin premix on a DM basis. The EI diet consisted of 34% endophyte-infected seed (Grande variety; 4.8 µg ergovaline g⁻¹ seed; 1.6 mg ergovaline kg⁻¹ mixed feed), 40% chopped bermudagrass hay, 16% oats, 4% soybean meal, 5% molasses, 0.5% limestone, 0.5% ammonium chloride, and 0.15% vitamin premix on a DM basis. The CP for both diets was 12% (DM basis) and TDN was 70%, which exceeded the NRC (1985) recommendations for percent nutrients in diet. Daily intake for each lamb was measured. Endophyte-infected fescue diet was fed at 3.0% BW (DM basis), but voluntary intake averaged 2.4% BW. So that intake was similar between treatments, the EF lambs were fed at 2.4% BW (DM basis). This resulted in an average daily intake of 30.3 µg ergovaline kg⁻¹ BW in EI fed lambs. Ergovaline in seed was determined by high-performance liquid chromatography (Rottinghaus et al., 1991).

Lambs were confined to individual raised pens and exposed to natural lighting. Feed refusals were collected, weighed, and animals were fed at 08:00 daily.
Rectal temperature and respiration rate were measured at 14:00 daily while animals rested quietly in pens. Respiration rate was determined by counting the number of breaths (girth movement or breath sounds) in 30 s and multiplying by 2. Temperature and humidity of the housing facility were recorded continuously using a temperature humidity recorder (Dickson THDx, Addison, IL). Blood was collected at 07:00 every Tuesday and Friday for collection of serum to determine concentrations of testosterone and prolactin (Tuesday only). Rams were weighed every 14 days.

All rams were transported every Tuesday at 07:30 to a handling facility for semen collection. Wool was sheared from scrotal area at start of study. Both scrotal circumference and scrotal skin temperature (Omega, model OS-610; Omega Engineering Inc., Stamford, CT) were measured at the widest point of scrotum before semen collection. Accessory glands were stimulated with an electro-ejaculator (Electroejac IV; Ideal Instruments/Neogen Corporation; Lansing, MI) at an automatic setting of 2 s intervals with 2 s rest, increasing voltage stimuli one volt at a time for up to two minutes or until ejaculation occurred. Semen from a single ejaculate was collected in a conical tube protected by a plastic sleeve, then placed in a 37 °C water bath. Percent sperm motility, percent abnormal sperm, and rate of forward movement were determined by a single technician without knowledge of treatments according to Sorensen (1979). Briefly, a live-dead stain (2% Fast Green and 0.4% Eosin Blue in phosphate buffer) was used to visualize percent abnormal sperm. Percent sperm motility was assessed on a drop of raw semen and from the number of live cells from the live-dead stain. Rate of forward movement was expressed on a scale of 1–4 (1 = no forward movement; 4 = fast forward movement).

Semen concentrations of spermatozoa were determined using an Animal Reproductive Systems (Model 543B, Chino, CA) densimeter (colorimeter) in the experimental mode. The sperm concentration of a composite sample of semen was initially determined by counting with a hemacytometer. The percent transmission was then determined for diluted semen samples ranging in concentration from 100 million up to 3.2 billion sperm ml⁻¹. The linear fit was then calculated for sperm concentration versus percent transmission. Semen samples were diluted 100:1 in physiological saline (at 37 °C), placed into 2 ml cuvettes and the percent transmission measured on duplicate samples. Sperm concentration ml⁻¹ was determined using the equation, 4347.130 — (50.338 × percent transmission) ($R^2 = 0.98$).

After 43 days of feeding, lambs were weighed and transported 151 km to the University of Arkansas Red Meat Abattoir. Feed and water were withheld from lambs overnight (approximately 12 h) before harvest. Lambs were rendered unconscious and insensitive to pain by captive-bolt stunning, and subsequently harvested according to industry-accepted procedures. Reproductive tracts were removed and individual organs (paired testicles, paired epididymis, pampiniform plexus, seminal vesicles, bulbourethral, ampulla) weighed. Liver, kidney, heart, and spleen were also weighed.

Serum was analyzed for testosterone in duplicate in two RIA assay runs (Diagnostic Products Corporation, Los Angeles, CA) according to Richards et al. (1999). Samples were organized randomly. Intra- and inter assay CV was 11 and 5%. Serum concentrations of prolactin were determined using RIA procedures of Moura and Erickson (1997) and Hockett et al. (2000). Sensitivity of the assay was 0.05 ng ml⁻¹ and intra and inter assay CV was 5.5 and 6%, respectively.

Data were analyzed by mixed models procedures of SAS (1996). Repeated measures were used for reproductive responses collected once or twice weekly and daily rectal temperature and respiration rate (Littell et al., 1996). Treatment and time were considered fixed effects and breed and animal within treatment were random effects. When interactions were significant, separation of means was conducted using the PDIFF option of LS means. The Day 1 values for semen concentrations of spermatozoa and rate of forward movement were used as a covariate in each analysis because of differences between treatment groups on Day 1. Therefore, the least squares means generated using the covariate analysis were presented.

Homogeneity of regression was used to examine effect of diet on the relationship between response variables (serum hormone concentrations, rectal temperature, or respiration rate) and day of feeding. This method compared error sums of squares between a mathematical model with time (one slope) or one with treatment and time (two slopes) using the GLM procedure (SAS, 1996). Day was tested to the order of significance. Similarly, the relationship between max-
imum daily ambient temperatures and the response variables, serum hormone concentrations, rectal temperature, or respiration rate, for each dietary treatment was examined. The order of significance was tested and determined to be linear.

3. Results

Least squares mean rectal temperature (diet × day, \( P = 0.14 \); Fig. 1A) and respiration rate (diet × day, \( P = 0.14 \); Fig. 1B) were similar between diets over the 6-week feeding period. On days in which maximal ambient temperature exceeded 22°C, rectal temperature and respiration rate were greater in EI fed rams. In fact, rectal temperature was greater in EI fed rams when regressing rectal temperature by daily high ambient temperature (\( y_{EF} = 39.0 + 0.05x, y_{EI} = 39.2 + 0.05x \), where \( y = \) rectal temperature in °C and \( x = \) ambient temperature in °C; \( P < 0.001; R^2 = 0.11 \)). Respiration rate was similar between diets at the lowest temperature, but increased at a greater rate as daily high ambient temperature increased in EI compared with EF fed rams (\( y_{EF} = −29.6 + 3.8x, y_{EI} = −66.1 + 5.8x \), where \( y = \) respiration rate in breaths min\(^{-1}\) and \( x = \) ambient temperature in °C; diet × day, \( P < 0.001; R^2 = 0.25 \)).

Scrotal skin temperature determined on days of semen collection was similar between treatments (\( P = 0.33 \)). Serum concentrations of prolactin were reduced in EI compared with EF fed rams (diet × day, \( P < 0.001 \); Fig. 2A) and were positively influenced by ambient temperature in EF, but not EI fed rams (\( y_{EF} = −4.5 + 0.50x, y_{EI} = 2.1 + 0.043x \), where \( y = \) serum concentrations of prolactin in ng ml\(^{-1}\) and \( x = \) ambient temperature in °C; \( P < 0.001; R^2 = 0.13 \)).

The CP and TDN in both diets exceeded that recommended by NRC (1985) on a percent nutrient in diet basis, but rams received less than the NRC recommended 4.0% DM intake per body weight because EI-fed rams refused anything more than 2.4% DM. Body weight loss between 0 and 36 days of feeding tended to be more for EI-fed rams compared with a slight gain in EF fed rams (−3.0 kg versus \( 0.51 \pm 1.2 \) kg; \( P < 0.07 \); diet × day, \( P < 0.02 \)). Live weight at slaughter was 59.9 ± 3.7 and 53.8 ± 3.7 kg for EF and EI fed rams (\( P = 0.26 \)). Weights of reproductive and vital organs were similar between EF and EI rams (data not shown).

Serum concentrations of testosterone were greater in EI fed rams with regression analysis (\( y_{EF} = 7.0 − 0.34x + [8.7 \times 10^{-3}]x^2, y_{EI} = 7.8 − 0.12x + [2.7 \times 10^{-3}]x^2 \), where \( y = \) serum concentrations of testosterone in ng ml\(^{-1}\) and \( x = \) day of feeding; \( P < 0.005, R^2 = 0.08 \)) but not the proc mixed analysis (\( P = 0.19 \), Fig. 2B). Similarly, there was a relationship between serum concentrations of testosterone and ambient temperature in that this circulating steroid increased between 16 and 20°C and decreased between 20 and 26°C (\( y_{EF} = −53.0 + 5.9x − 0.15x^2, y_{EI} = −42.6 + 4.9x − 0.12x^2 \), where \( y = \) serum concentrations of testosterone in ng ml\(^{-1}\) and \( x = \) ambient temperature in °C; \( P < 0.004; R^2 = 0.10 \)).

Fig. 1. Least squares means of rectal temperature (A; diet × day, \( P = 0.14 \)) and respiration rate (B; diet × day, \( P = 0.14 \)) for rams fed a diet free of endophyte-infected tall fescue seed (open circles; EF; \( n = 8 \)) or endophyte-infected seed (closed circles; EI; \( n = 8 \)) diet during Days 1 through 43 of feeding (Day 1 = first day of feeding). Right hand y-axis represents daily high ambient temperature.
Fig. 2. Least squares means of serum concentrations of prolactin (A), testosterone (B), or rate of forward movement of spermatozoa (C) for rams fed a diet free of endophyte-infected tall fescue seed (open circles; EF; n = 8) or endophyte-infected seed (closed circles; EI; n = 8) diet (Day 1 = first day of feeding; diet × day, \( P < 0.001 \)).

Difference between diets for a particular day indicated by a single asterisk (\( P < 0.05 \)) or double asterisk (\( P < 0.01 \)).

Semen concentrations of spermatozoa were similar between diet groups (\( P = 0.11 \)) and tended to increase over time in both treatments (\( P < 0.09 \)). Spermatozoa concentrations in EF-fed rams tended to increase to a greater extent during the final weeks of study when comparing slopes of dietary groups (\( y_{EF} = 0.81 + 0.044x \), \( y_{EI} = 1.3 + 0.02x \), where \( y = \) spermatozoa concentration \( 10^9 \) ml\(^{-1} \) and \( x = \) day of feeding; \( P < 0.10; R^2 = 0.15 \)). Scrotal circumference was similar between diets on all days and decreased between Day 1 and Day 43 for both groups (EF: Day 1, 32.8 cm, Day 43, 31.6 ± 0.9 cm, \( P = 0.05 \); EI: Day 1, 32.9 cm, Day 43, 30.2 ± 0.9 cm, \( P < 0.001 \)).

Volume of semen collected (1.28 ml versus 1.32 ± 0.17 ml; \( P = 0.84 \)) and percent sperm motility (68.5% versus 74.0 ± 9.7%; \( P = 0.69 \)) were similar between EF and EI diets and increased from Day 1 (0.78 ± 0.17 ml; 49.7 ± 8.6%) to Day 43 (2.21 ± 0.18 ml; 90.0 ± 8.7%) of the feeding period (\( P < 0.001 \)). Percent abnormal sperm was similar between EF and EI diets (8.2% versus 7.3 ± 1.1%; \( P = 0.55 \)) and decreased from Day 1 (9.7 ± 1.3%) to Day 36 (5.8 ± 1.2%; \( P = 0.002 \)). Rate of forward movement of spermatozoa tended to increase at a greater rate between Days 8 and 29 in EF compared with EI fed rams (proc mixed: diet × day, \( P = 0.08 \); Fig. 2C; regression: \( y_{EF} = 2.3 + 0.048x \), \( y_{EI} = 3.4 + 0.0019x \), where \( y = \) rate of forward movement and \( x = \) day of feeding; \( P < 0.02; R^2 = 0.07 \)).

4. Discussion

This is the first report describing reproductive responses of ruminant males in response to feeding an endophyte-infected tall fescue diet in which the ergovaline consumption and ambient temperature was characterized. Signs of fescue toxicosis in these animals included increased rectal temperature and respiration rate consistent with previous reports (Oliver, 1997; Burke et al., 2001) and feed intake was limited to 2.4% BW. Decreased feed intake is typical of animals grazing EI fescue (Hemken et al., 1981; Paterson et al., 1995). To minimize confounding effect of lower intake, the intake of EF-fed lambs was restricted to that of the EI-fed group.

Scrotal circumference and semen characteristics were within normal ranges for both groups of rams.
Evans et al. (1988) observed little difference in reproductive development between young bulls fed endophyte-free or infected tall fescue hay. In the current study, continued feeding of infected fescue was associated with a static concentration of spermatozoa compared with increased concentrations of spermatozoa over time in rams fed a control diet. In addition, rate of forward movement tended to be greater in rams fed the diet free of infected fescue. Schuenemann et al. (2005) determined that fertilization could be impaired, because cleavage rate of oocytes fertilized by bulls fed ergotamine was reduced. These responses follow the widely observed trend for reduced reproductive performance in fescue fed ruminants.

Increased testosterone in rams fed EI seed was unexpected. Testosterone and prolactin were reduced in rams administered bromocryptine (Regisford and Katz, 1993). Serum concentrations of testosterone were similar between control bulls and those fed infected fescue (Evans et al., 1988). In models examining a stress response, rams administered endotoxin (Walgren et al., 1989) or dexamethasone (Tsantarliotou et al., 2002) experienced a decrease in circulating testosterone. In ruminant females, progesterone decreased in testosterone may reflect different clearance rates compared with increased concentrations of spermatozoa over time in rams fed a control diet. In addition, rate of forward movement tended to be greater in rams fed the diet free of infected fescue. Schuenemann et al. (2005) determined that fertilization could be impaired, because cleavage rate of oocytes fertilized by bulls fed ergotamine was reduced. These responses follow the widely observed trend for reduced reproductive performance in fescue fed ruminants.

Decreased clearance rate of steroid hormones by the liver could be masked in more severe incidences of fescue toxicosis. If synthesis or secretion of steroids decreases, an increase in circulating steroid hormones would not be observed. In addition, total cholesterol, a steroid precursor, decreases in cattle in association with consumption of tall fescue (Stuedemann et al., 1985).

Other differences among reproductive responses in reported studies could be attributed to differences in intake of ergot alkaloids. Prolactin did not decline in bulls exposed to ergot alkaloids (Evans et al., 1988), but ergovaline levels of infected hay were not determined. Concentrations of ergovaline in infected tall fescue hay at the current research station have been as little as 0.14 μg kg⁻¹ (DM basis; Burke, unpublished data) and can range between 0.1 and 6.0 μg kg⁻¹ in seed and forage (Belesky et al., 1988; Hill et al., 1991; Porter, 1995). In the Evans et al. (1988) study, if the ergovaline concentration of the hay fed to the bulls was as little as 0.1 μg kg⁻¹, bulls would have received between 1.7 and 2.9 μg ergovaline kg⁻¹ BW, if animals weighed between 100 and 350 kg and consumed feed at a level of 3.5% BW day⁻¹. Mizinga et al. (1992) noted no changes in serum concentrations of prolactin while heifers were fed less than 9.75 μg ergovaline kg⁻¹ BW day⁻¹. Fescue toxicosis may not be induced in animals exposed to lower levels of ergot alkaloids or induction of toxicosis may be dependent on environmental stressors at these low levels. In the current study, serum concentrations of prolactin were reduced on three of six of the days determined in rams fed 30.3 μg ergovaline kg⁻¹ BW daily. Because rams were not exposed to heat stress for much of the study, signs of fescue toxicosis may have been lower than expected.

On the other hand, differences in ambient temperature among reported experiments could contribute to whether prolactin and reproductive responses were reduced in endophyte-infected fescue fed ruminants. Concentrations of prolactin in EF-fed rams were increased at higher ambient temperatures (Days 8 and 22) in the current experiment, but similar to EI-fed rams at cooler temperatures. Others (Hooley et al., 1979; Walker et al., 1990) observed increased prolactin in heat-stressed ewes. Respiration rate and rectal temperature were greater in the EI fed rams when the ambient temperatures exceeded 22°C in the current study. Similarly, respiration rate and rectal tempera-
tures were greater in heat stressed cattle, but not cattle at thermoneutral temperatures that were fed infected fescue (Hemken et al., 1981; Burke et al., 2001). Lack of differences in circulating prolactin, rectal temperature, and respiration rate on cooler days between the two groups of rams further indicates that fescue toxicosis in EI-fed rams was less prominent on cooler days. Changes in reproductive responses in male ruminants experiencing heat stress have not been reported other than severe heat stress leading to infertility (American Sheep and Industry, 2003). Gonadotropin secretions were altered in heat stressed dairy cows (Wolfenson et al., 2000), which suggests that changes in gonadotropin function could occur in males, leading to reduced fertility. Speculatively, rate of forward movement of spermatozoa could have been slowed by increased scrotal temperature, as body temperature was increased in EI-fed rams.

It is not surprising that weights of vital and reproductive organs were similar between dietary treatments because live weight was similar and the short experimental duration. Carcass characteristics were also similar (data not shown). Because feed intake was similar between treatment groups, changes in growth were not expected.

5. Conclusion

In summary, higher ambient temperatures led to increased serum concentrations of prolactin, rectal temperatures, and respiration rates in rams fed infected fescue, not observed when temperature was less than 23 °C. Continued feeding of infected fescue tended to lead to a reduced number of spermatozoa produced and rate of forward movement, compared with EF feeding, which could lead to reduced conception rates in the female during the 6-week breeding period.

Ruminants raised in regions that grow tall fescue will likely encounter periods of exposure to infected tall fescue because of the success of this cool season grass in a grazing program. While eradication of tall fescue is not necessary or practical to maximize conception rates, proper management of breeding animals is necessary. Reduction in male fertility associated with consumption of endophyte-infected tall fescue may have occurred in the current study. Long term studies on male ruminants exposed to endophyte-infected tall fescue are necessary to assess maximal impact on fertility.

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