

NUTRITIONAL INFLUENCES ON REPRODUCTION: EFFECTS OF ENDOPHYTE-INFECTED TALL FESCUE



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Introduction

Tall fescue, a cool-season perennial grass, is one of the most commonly grown forages for over 8.5 million cattle in the United States [Hoveland, 1993]. Cattle suffering from fescue toxicosis experience decreased feed intake and performance, elevated respiration rate and body temperature, rough hair coats and necrosis of the extremities (tail, hooves, and ears) due to loss of circulation [Paterson et al., 1995]. Endocrine and reproductive effects of fescue toxicosis in cattle include decreased calving rate [Porter and Thompson, 1992] and pregnancy rates [Gay et al., 1988; Brown et al., 1992; Seals et al., 2005], reduced circulating concentrations of hormones such as cortisol, prolactin (PRL; [Porter, 1995]), progesterone (P₄; [Mahmood et al., 1994; Jones et al., 2003]), and LH [Porter and Thompson, 1992; Mahmood et al., 1994]. This toxicosis results in estimated losses to the United States beef industry of \$609 million annually due to lowered conception rates and depressed body weight gains [Hoveland, 1993; Paterson et al., 1995].

We have made considerable progress in “identifying the window” in timing of reproductive loss associated with grazing infected tall fescue. We also know that addition of clover to our pastures will help reproductive performance in cattle, as does the addition of supplemental feeding of grain (etc.). This supplementation with clovers and grain is thought to have a “diluting” effect on the toxic component of tall fescue grass. In technical terms, the assumed “bad” component of tall fescue infected with the endophyte, *Neotyphodium coenophialum*, is an alkaloid known as “ergovaline” (produce by the endophyte) that negatively affects performance of the animal but plays a beneficial role on the hardiness of the grass [Hill et al., 1991; West et al., 1993; Ball et al., 1996; Thompson et al., 1999]. However, recent research by Hill et al. [2001] indicates that transport of the ergopeptine alkaloid “ergovaline” across ruminal gastric tissue is low as compared to the simple ergoline alkaloids such as lysergic acid; thus, suggesting other alkaloids may play a larger role in tall fescue toxicosis.

So with all this said, the tall fescue research team at the University of Tennessee has focused their research attention on determining “how” and “when” the grazing of endophyte-infected tall fescue (E+) affects reproduction in cows and bulls. We performed these studies by either grazing tall fescue pastures (E+ or MaxQ, non-toxic endophyte, NTE) or by using a synthetic compound called ergotamine tartrate (referred to as ERGOT) to simulate the negative effects of ergovaline since ERGOT was commercially available, presented the same signs of tall fescue toxicosis, and we could control the nutritional status of the animal (thus removing nutrition from the equation as it relates to reproduction). Now to the reproduction part, we first wanted to know **when** consumption of endophyte-infected tall fescue had a negative effect on reproduction. There are several different periods of concern when looking at reproduction in a beef cattle setting, so we

broke these time periods into different stages beginning with (1) the effect on the bull, (2) late pregnancy losses in the cow, (3) losses due to hormonal changes before estrus (heat), and (4) embryonic or uterine losses immediately following estrus.

Effects on the Bull

Few studies have focused on the beef bull, as related to fescue toxicosis, which were conducted with sufficient numbers or replicates to draw conclusions. Studies in mice [Zavos et al., 1988] and dairy bulls [Evans et al. 1988] had suggested a detrimental effect (mice) or no effect (Holstein bull calves) when consuming tall fescue seed or hay, respectively. Alamer and Erickson [1990] reported that yearling beef bulls grazing E+ tall fescue contained fewer Sertoli cells, suggesting impaired testicular function.

Analysis of data from a three-year project performed at Highland Rim Research and Education Center with 96 yearlings beef (Angus and Gelbvieh) bulls presented some interesting findings. Year 1 [Schuenemann et al., 2005a] of the experiment involved yearling bulls receiving a control diet of corn silage supplemented with soybean meal (n=8) or a treated diet consisting of corn silage supplemented with soybean meal and “ergotamine tartrate (n=8) for a period of 224 days (November through June). This study was performed to control for the detrimental effect of tall fescue consumption on nutrition or weight loss. Again, feeding of ERGOT allows for us to control for the “nutrition factor” by regulating feed consumption and enables us to focus on the effects of the alkaloid on male fertility. Years 2 and 3 [Schuenemann et al., 2005b] utilized two sets of yearling beef bulls to evaluate the effect of actually grazing (experimental period of 224 days) non-toxic tall fescue (NTE; MaxQ; n=10/year) or endophyte-infected (E+) tall fescue with (n=10/year) or without clover (n=20/year). Body weights, blood samples, forage samples and rectal temperatures were collected every 2 weeks. Every 60 days, scrotal circumference was recorded and semen collected for evaluation of motility and morphology. Testicular core temperatures were measured immediately before semen collection at the beginning of May and the end of June each year. Semen was extended immediately following collection and returned to the laboratory for evaluation through our in vitro fertilization program to determine fertilization potential and subsequent embryo development.

In brief, bulls consuming the diet supplemented with ergotamine tartrate had similar weight gains to control bulls as desired for the study. Scrotal circumference and semen motility and morphology were similar between treated and control bulls but fertilization potential (cleavage) was reduced (Table 1) in ERGOT bulls compared to controls. Subsequent development of embryos that cleaved was similar between treatments. However, testicular core temperatures were reduced in ERGOT bulls (Figure 1) compared to controls even though rectal temperatures were elevated suggesting a vasoconstrictive effect of consuming ergotamine tartrate on the testis.

During Years 2 and 3, yearling beef bulls grazing E+ pastures without clover performed poorly (average daily gain) but no differences were noted in scrotal development or semen quality (as evaluated during a breeding soundness exam) in May and late June

compared to bulls grazing NTE (MaxQ) pastures. However, the fertilization ability or potential (ability to cleave) of the semen was reduced (Table 2) in bulls grazing E+ Table 1. Ability of sperm collected from bulls fed a control or ergotamine tartrate-supplemented diet to fertilize bovine oocytes [Schuenemann et al., 2005a].

TRT	REP (n)	COC (n)	PZ (n)	Cleav (%)	Blast (%)
CON	2	200	169	69.2±3.3 ^a	22.2±3.1
ERGOT	2	200	143	51.1±3.3 ^b	22.0±3.1
P-value				0.001	0.96
Lab Con	2	100	86	74.4	43.3

^{a, b} Least squares means differ within a column

Reps: total number of replications per bull (Replicate 1, May 5th; Replicate 2, June 28th)

COC: cumulus oocyte complexes

Cleav: number of putative zygotes cleaved

Blast: blastocyst; percentage of cleaved embryos developing to blastocyst

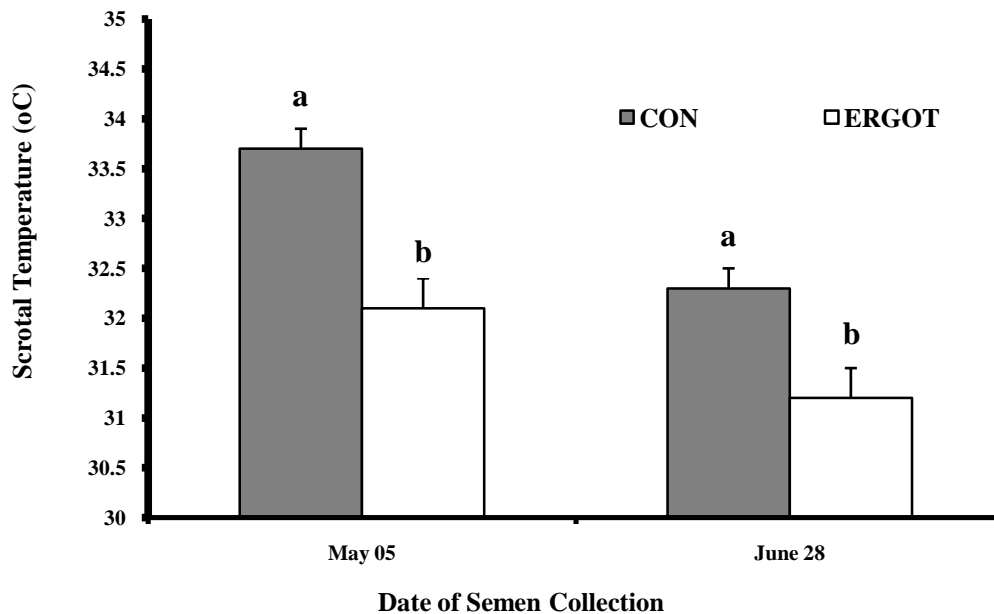


Figure 1. Scrotal thermography measured at the time of semen collection on May 5th and June 28th. Scrotal temperatures recorded immediately before semen collections were lower in bulls administered ergotamine tartrate (ERGOT) compared to control (CON) animals (^{a, b} Least squares means differ within treatments; $P < 0.05$), [Schuenemann et al., 2005a].

pastures without clover compared to NTE pastures. Again, rectal temperatures were elevated but with a reduction in testicular core temperatures (as measured by thermography) in bulls grazing E+ tall fescue pastures. Addition of clovers to the E+ pastures improved gain performance but fertilization potential of semen was not determined. Additional studies are ongoing investigating pregnancy rates associated with

artificial insemination of heifers and penetration rate of semen from bulls grazing either E+ or NTE pastures.

Table 2. Ability of sperm collected from bulls grazing tall fescue pastures to fertilize bovine oocytes [Schuenemann et al., 2005b].

Variables	NTE	E+	Lab Control
Rep (n/yr)	2	2	2
COC (n)	850	873	278
Cleav (%)	84 ± 2.4 ^a	73.5 ± 3.1 ^b	81 ± 3.8
Blast (%)	30.1 ± 4.7	32.4 ± 5.5	30 ± 5.5
Nuclei (#)	76.5 ± 4.5	72.9 ± 4.8	73.6 ± 5.2

^{a, b} Least squares means differ within a row, $P < 0.05$

COC: cumulus oocyte complexes

Cleav: number of putative zygotes cleaved

Blast: blastocyst; percentage of cleaved embryos developing to blastocyst

Nuclei #: total number of cells in blastocyst after fixation and Hoechst staining

Future studies needed on the male side include determining the effect of E+ tall fescue on reproduction parameters of MATURE bulls and sperm defects associated with fescue toxicosis and reduced fertilization ability. Another potential study of interest includes evaluation of tall fescue effects on bulls that have been removed from these pastures for a period of time then re-introduced back on tall fescue pastures during the breeding period.

Effects on the Cow

On the female side, it was observed that late pregnancy losses *were not occurring* at a higher rate in cows and heifers grazing E+ tall fescue as compared to animals grazing endophyte-free pastures. Heifers grazing infected tall fescue that were confirmed 30 days pregnant by ultrasonography stayed pregnant at an acceptable rate AND that the reproductive loss had already occurred by 30 days [Waller et al., 2001]. Thus, we deleted *late pregnancy losses* as a concern in cows grazing endophyte-infected tall fescue. Similar results have been reported by several laboratories suggesting that reproductive losses occur early during pregnancy.

Next, we wanted to determine if changes in reproductive hormones or the follicle that produced the egg (oocyte) that would result in pregnancy differed in animals receiving ergotamine tartrate (ERGOT) to simulate fescue toxicosis [Seals et al., 2005]. In brief, pregnancy rates determined at 30 days postbreeding were reduced in cattle receiving ERGOT without any changes in reproductive hormones (Figure 2) or alterations in growth of the ovulatory follicle. Contrasting results have been reported concerning follicular development and alterations in reproductive hormones [Burke et al., 2001a; Burke et al 2001b; Jones et al., 2004] with others in agreement [Fanning et al., 1992]. Thus, in our laboratory with ergotamine tartrate, effects of fescue toxicosis were evident on pregnancy but not on any reproductive events that occurred immediately before breeding.

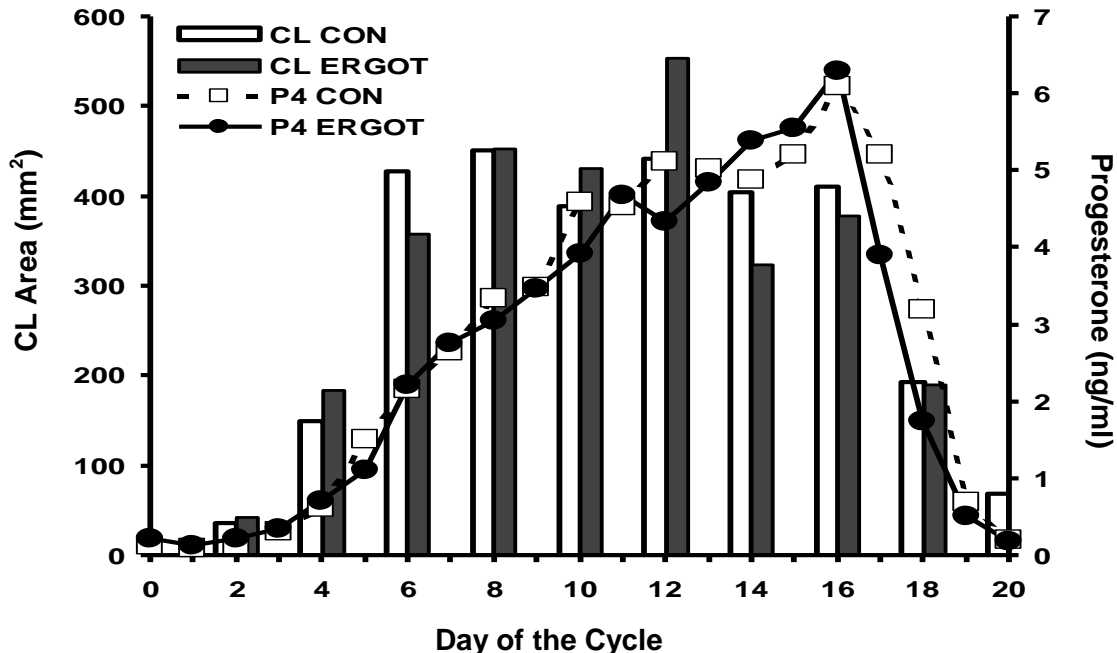


Figure 2. Area of the corpus luteum overlayed with progesterone concentrations during the ultrasonography period [Seals et al., 2005].

Next, we asked the question of whether the uterus was capable of maintaining a pregnancy when a good embryo was transferred into the reproductive tract on day 7 after estrus. In short, was fescue toxicosis causing a problem within the uterus? Rahe et al. [1991] suggested that losses were occurring after d 7 of pregnancy (following embryo transfer) but left several unanswered questions. We observed that pregnancy rates were acceptable following transfer of frozen-thawed embryos (collected from cows not exposed to E+ tall fescue) in both groups of heifers that served as either controls or those receiving ERGOT [Schuenemann et al., 2005c]. Even though rectal temperatures were elevated, pregnancy rates were 50% in ERGOT heifers following embryo transfer. Thus, we deleted the *uterine environment after day 7* as a factor in reduced pregnancy rates during fescue toxicosis in heifers receiving ergotamine tartrate.

So when is fescue toxicosis affecting reproduction in the cow? The next experiment provided us a “smaller time frame”. Heifers were allotted into their respective treatment groups: receiving or not receiving ERGOT to simulate fescue toxicosis [Schuenemann et al., 2005c]. Estrus was synchronized and heifers (non-FSH stimulated) inseminated with semen from the same bull. Seven days later, single embryo recoveries were performed and embryos graded (evaluated) for quality and development. Embryos from heifers receiving ERGOT were reduced in quality and development (Figure 3). In other words, they were poor compared to our control embryos. Furthermore, recovery rate tended to be reduced in heifers supplemented with ergotamine tartrate.

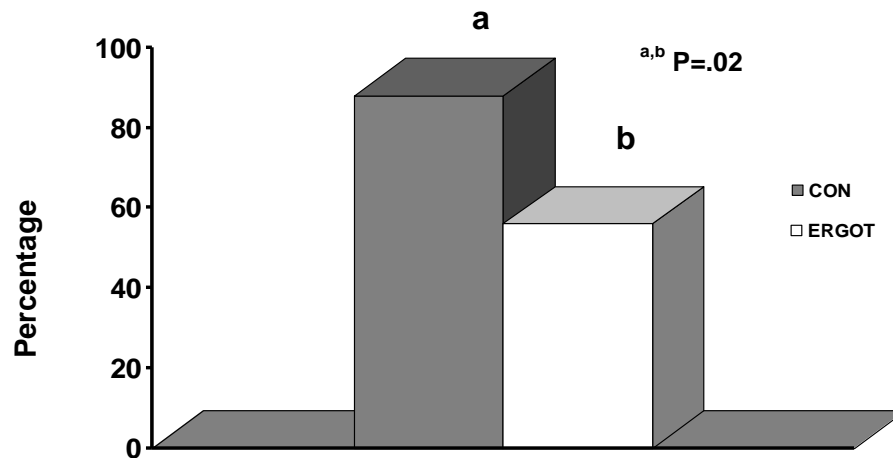


Figure 3. Development (percentage of embryos that had attained the compacted morula or blastocyst stage) of embryos recovered on day 7 after estrus in heifers consuming ergotamine tartrate to simulate tall fescue toxicosis or serving as controls [Schuenemann et al., 2005c].

Summary

So what does this all mean? Briefly, fescue toxicosis affects either the growing oocytes (egg) or the early embryo while still in the oviduct on the female side. Add in the effects of tall fescue on the sperm and we can understand why fertility is reduced. Throw in elevated temperatures during the summer months (June and July) with little (or no) clover and we could see a “reproductive wreck”. What we can suggest in terms of management is that we can remove cows from fescue for 30 days before and after breeding and see no effect on pregnancy rates (is this practical?). More practical would be to have your cows calve early (if spring calving) and get them exposed to the bull before the hot summer months occur (the same with your heifers). We intend to perform additional studies to determine if we can manage our females differently around the time of breeding (additional feeding, change pasture locations and types, etc.) to improve pregnancy rates when grazing infected tall fescue.

Another factor to keep in mind would include the possibility that you have selected, over time, animals that will tolerate E+ tall fescue. You have culled those heifers that did not grow well. You have sold those cows that would not get pregnant. Has your herd adapted to grazing tall fescue? What about the bull? Will a bull that has not been exposed to tall fescue in his lifetime work on your farm? All the animals used in the above studies had been born and raised on the farm where the research was performed and consumed E+ tall fescue (with clover) their entire life. So have your animals been selected for adaptation? I’ll leave that question for the genetic researchers!

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