



Bulls grazing Kentucky 31 tall fescue exhibit impaired growth, semen quality, and decreased semen freezing potential

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ABSTRACT

Serum prolactin (PRL) and testosterone concentrations, body weight, body composition, semen quality, and semen freezing potential for bulls grazing the toxic tall fescue (*Lolium arundinaceum* [Schreb.] Darbysh. = *Schedonorus arundinaceum* [Schreb.] Dumort.) cultivar Kentucky 31 (E+) compared with a novel endophyte cultivar lacking ergot alkaloids (E-) were evaluated. Angus bulls were allotted to treatment (Day 0) and grazed E+ or E- for 155 days. Treatment-by-day interaction was significant ($P < 0.05$) for serum PRL concentrations with E+ treated bulls exhibiting reduced PRL values compared with E- control bulls, but no differences were observed for serum testosterone concentrations ($P > 0.05$). Further, bulls on the E+ treatment exhibited decreased total gain, average daily gain, and body weight by Day 140 ($P < 0.05$) compared with the E- bulls. Rump muscle depth was lower because the treatment in bulls grazing E+ compared with E- ($P < 0.05$) and intramuscular fat in the E- bulls compared with the E+ group was higher by Day 155 ($P < 0.05$). Analysis of ejaculates showed significant treatment \times day effects for sperm concentration with lower values observed for bulls on the E+ treatment ($P < 0.05$). The percent normal morphology was reduced in ejaculates from E+ bulls compared with E- bulls ($P < 0.05$), and the difference was due to an increase in abnormal sperm present in the E+ ejaculates from Day 84 to 140 ($P < 0.05$). In addition, spermatozoa motility and progressive motility were decreased on thawing in semen samples from E+ bulls compared with E- bulls ($P < 0.05$).

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1. Introduction

Tall fescue (*Lolium arundinaceum* [Schreb.] Darbysh. = *Schedonorus arundinaceum* [Schreb.] Dumort.) is present on approximately 16 million ha in the Mid-Atlantic and Southern regions of the United States [1] and

serves as a forage for approximately 8.5 million cattle, making this species the dominant, cool-season, perennial grass in the region. The vast majority of this forage is infected with the endophyte, *Neotyphodium coenophialum*. The grass exists in a mutualistic relationship with the ergot alkaloid-producing endophyte that confers disease, drought, grazing tolerance, and insect resistance to tall fescue [1]. However, ergot alkaloids negatively impact growth and/or reproductive performance of animals grazing tall fescue containing the wild-type endophyte.

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Accumulation of the ergot alkaloids within the animal's system results in the syndrome known as fescue toxicosis, which is characterized by rough hair coat, increased core body temperature, decreased blood flow to body extremities, and poor growth and reproductive performance [2]. Previous estimates for lost beef production due to fescue toxicosis were \$600 million [1], and more recent reviews estimate that total to be approaching \$1 billion lost per year for cattle and small ruminants [2,3].

The most studied aspects of fescue toxicosis are the effects on animal performance [4–7]. However, there is only limited documentation of the effects of fescue toxicosis on reproductive function [8–11] and very little, if any, of fescue toxicosis on bovine male reproduction [12–16]. In an effort to begin filling this gap, our previous research evaluated the effects of fescue toxicosis on semen characteristics when bulls were fed a constant amount of ergot alkaloid in conjunction with a high-concentrate ration to delineate effects of the ergot alkaloid from possible effects of reduced body condition and plane of nutrition [16]. Little, if any, effects were noted on breeding soundness examinations (BSE) or semen characteristics. The objectives of this study were to determine the effects of grazing endophyte-infected ergot alkaloid-producing Kentucky 31(KY31) or nonergot alkaloid-producing novel endophyte pasture on body composition, semen quality, and semen freezing potential for yearling beef bulls.

2. Materials and methods

2.1. Experimental design

All animal research was approved by the Clemson University Institutional Animal Care and Use Committee (IACUC protocol #ARC2010–68). All reagents were purchased from Sigma Scientific (St. Louis, MO, USA) unless stated otherwise. Angus bulls ($n = 21$) aged between 13 and 16 months exhibiting scrotal circumference (SC) of 30 cm or greater, a minimum of 30% motility, and 70% normal sperm BSE were stratified by body weight (BW) and body condition score (BCS) and allotted to one of the two treatments. Two bulls did not meet these minimum requirements and were not used for further SC measurements or semen analysis but were maintained on study for growth and body composition estimates. Stocking rates were one bull per 0.4 hectares for bulls grazing novel endophyte non-toxic fescue (E-) and 1.1 bulls per acre for the bulls grazing the ergot alkaloid producing KY31 tall fescue (E+). Bulls were rotationally grazed using two 4.0-hectare paddocks. All bulls were evaluated at specific intervals for reproductive and growth parameters across a 155-day grazing period. The study duration was designed to monitor bulls through two full spermatogenic cycles.

2.2. Treatment

Dietary treatments consisted of grazing the ergot alkaloid-producing KY31 or a nonergot alkaloid-containing tall fescue possessing a novel endophyte. The enzyme-linked immunosorbent assay test for ergot alkaloids (Agrinostics, LTC. Co, Watkinsville, GA, USA) was conducted on 50 tillers per pasture, and the E+ pasture exhibited a 98% infection

rate. Two weeks before the start of the study, all bulls were adjusted to a forage diet by grazing E- pasture. At the start of the test (Day 0), bulls were weighed, BCS was evaluated, SC and semen quality were assessed, stratified, and allotted to E+ or E- treatments and remained on treatment for 155 days (April 2012 to August 2012).

2.3. Blood collection and RIA

Blood samples were obtained via the coccygeal vein at the start of test and on Days 35, 84, 114, and 140 and assayed for serum prolactin (PRL) and testosterone. Blood was allowed to clot and placed at 4 °C overnight, and serum was harvested by centrifugation at $\times 2000g$ for 15 minutes at 4 °C. Serum was placed in vials and stored at -20°C until used in RIA. Prolactin assays were performed by the F. Neal Schrick laboratory as previously described [17] with mean interassay and intra-assay coefficients of variation of 9.7% and 6.0%, respectively. Concentrations of testosterone were determined in a single assay using the Coat-A-Count testosterone RIA with a sensitivity of 6 ng/dL (Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA) with an intra-assay coefficient of variation of 6.3%. The assay was validated by generating two composite pools of bull plasma containing 494 and 855 ng/dL of testosterone, and recovery was $98.1 \pm 1.7\%$, $99.9 \pm 2.9\%$, and $101.4 \pm 2.5\%$, respectively. Additionally, when three different dilutions (one, two, and threefold dilutions) of each of the pooled samples were assayed and the recovered values were plotted, the slopes of the inhibition curves were similar to that of the standard curve ($P = 0.89$).

2.4. Semen evaluation

Bulls were restrained in standard animal handling chutes and subjected to electroejaculation using the Pulsator IV electroejaculator (Agtech, Manhattan, KS, USA) on the preprogrammed collection mode. The ejaculate volume was recorded, and semen quality parameters were estimated using a computerized sperm-quality analyzer (SQA-Vb; A-Tech, Los Angeles, CA, USA). Each sample had computerized sperm-quality analysis performed in duplicate, and if motility and/or morphology were below 30% and 70%, respectively, a second collection was obtained within 7 days of the first sample. If both samples were below 30% and 70%, respectively, the highest rated collection for the bull was used in subsequent statistical analysis. Parameters evaluated by computerized sperm-quality analysis were as described by Stowe et al. [16]. In addition, each ejaculate was subjected to a manual morphologic examination by standard staining and microscopic evaluation. All samples were independently evaluated by two technicians.

Bulls failed BSE if their semen samples from both collections exhibited less than 30% motility, less than 70% normal morphology, or an SC lower than recommended for their respective age range using the 1993 Society for Theriogenology guidelines for BSE [18].

2.5. Semen extension and freezing

Semen collection was conducted on bulls grazing E+ pasture ($n = 5$) and bulls grazing E- pasture ($n = 7$) on Day

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