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Assessment of semen quality and fertility in young growing beef bulls exposed to ergot alkaloids

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ABSTRACT

The objective of this study was to evaluate semen quality and fertility in beef bulls grazing the ergot alkaloid (EA) producing tall fescue cultivar, Kentucky 31 (KY31), compared to a novel endophyte (NE) cultivar lacking EA. Two studies were conducted over a 3-year period. In studies 1 ($n = 10$; ages ≥ 24 mo) and 2 ($n = 53$ over two years; ages 12–16 mo), Angus (AN) bulls were stratified by body weight (BW), body condition score (BCS), and scrotal circumference (SC), and then allotted to graze either KY31 or NE for 56 days. Semen samples were collected, and BW, BCS, and SC were evaluated at the start of treatment (TRT) on day (d) 0 and every 28 days to the end of each study. In addition, blood samples were collected on d 0 and every 28 days for assessment of circulating prolactin (PRL) levels in study 2. On d 56, for both studies, semen from bulls ($n = 2$ per treatment in study 1 and $n = 4$ per treatment in study 2) with similar and acceptable quality were extended, kept at 19° C, and used for timed artificial insemination (TAI) of primi- and multiparous AN and AN- crossbred females. Pregnancy was evaluated at 35 and 90 days post-TAI via transrectal ultrasonography to determine pregnancy rates. Serum PRL concentrations showed a TRT by d effect ($P \leq 0.05$), with values for bulls grazing KY31 decreased on d 28 and d 56 of grazing compared to NE. In studies 1 and 2, bull BW and BCS were affected by d ($P \leq 0.05$), but not by TRT. No TRT or TRT by d effect on semen quality was observed in either study; however, d impacted both velocity and concentration in study 2 ($P \leq 0.05$). In study 1, TAI pregnancy rates at 35 days post-TAI were lower ($P \leq 0.05$) in the group inseminated with semen from bulls grazing KY31; however, in study 2, pregnancy rates did not differ due to treatment 35 post-TAI ($P > 0.05$). Grazing KY31 negatively impacted serum PRL concentrations, supporting previous observations; however, consumption of KY31 had no effect on growth or semen quality of AN bulls ranging from 12 to $\geq$24 mo of age. Furthermore, fertility data is inconsistent between studies and requires further investigation.

1. Introduction

Tall fescue is a cool season forage commonly used in the southeastern United States, with the predominant cultivar being Kentucky 31 (KY31) [1–5]. The KY31 cultivar is easy to establish, tolerates poor management, and adapts to a wide range of soil types [6,7]. Further, the plant is drought [8] and insect resistant, and can tolerate heavy grazing pressure. Many of these positive traits exist in KY31 due to the cultivar being infected with the endophyte, *Epichloë coenophiala* [9]. The endophyte produces ergot alkaloids [10], conferring drought and grazing tolerance [8,11] and disease and insect resistance [12,13] to the plant. Unfortunately, ergot alkaloids (EA) negatively impact animal growth [14–23], but the effect of fescue toxicosis on bovine reproduction is less clear, with studies indicating either reduced [24–27] or no effect [18,19] of ergot alkaloid consumption on reproductive performance.

As animals graze KY31, EA enter the animal's system and can alter physiology by binding to neuropeptide receptors [28–34]. Cattle physiological responses shown to be affected by EA consumption include vasoconstriction [35,36], elevated core body temperature [23,26,37–39], and depressed serum prolactin (PRL) concentrations [23,27,39–42]. The amalgamation of EA consumption results in animals suffering from the syndrome known as fescue toxicosis, in which symptoms such as rough hair coat,

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increased core body temperature, vasoconstriction, reduced weight gain and growth, and possible decreased reproductive efficiency [43,44] are displayed. Recent estimates on the loss of animal production in the region known as the "fescue belt", which includes approximately 16 million hectares within the mid-Atlantic and southern portions of the U.S., project losses approaching $1 billion per year for cattle and small ruminants [43].

Limited information regarding the impacts of fescue toxicosis on bovine male reproductive performance exists [41,42,45,46]. Moreover, the studies examining semen quality of bulls consuming diets with or without EA are contradictory. It has been reported that consumption of EA can reduce sperm concentration in ejaculates [42], sperm motility [46], percentage of normal sperm [42,46], and sperm morphology [42,46]. In addition, it has also been reported that consuming EA has no effect on semen quality [26,38,41]. Regardless, only two studies have attempted to assess male fertility of bulls consuming EA [26,38], and both reported a reduction in the percentage of cleaved embryos in IVF when using semen obtained from bulls consuming rations containing EA. More information on the impact (if any) of EA on bull semen quality and fertility is required; unfortunately, several reports have documented that semen obtained from bulls consuming EA does not survive normal semen freezing procedures well [42,47]. Fertility comparison of semen compromised during the freezing process would not be valid. Therefore, a fertility study would need to assess semen of similar quality in AI prior to freezing. The objective for this study was to compare semen quality and fertility of control bulls or bulls consuming EA using fresh extended semen, bypassing the issues of semen freezing observed when bulls consume EA.

2. Materials and methods

2.1. Experimental design

All experimental procedures were approved by the Clemson University Institutional Animal Care and Use Committee (AUP # 2014-60). Bulls used in this study were maintained at the Simpson Research and Education Center, Pendleton, SC. All cows used in this study were maintained at the Edisto Research and Education Center, Blackville, SC.

All reagents were purchased from MOFA Global (Verona, WI) unless stated otherwise. Study 1 was conducted to assess semen quality and fertility of Angus (AN) bulls ($n = 10$; ages ≥ 24 mo) in the presence or absence of EA using a forage ration. Bulls were purchased from breeders in North Carolina and South Carolina, which did not utilize EA producing tall fescue varieties in their production program. Bulls were maintained on rations that did not contain EA producing tall fescue varieties at Simpson Research and Education Center and prior to the study were adjusted to a total forage ration lacking EA for 14 days prior to the initiation of treatment (TRT), and were then subjected to grazing toxic Kentucky 31 (KY31) or nontoxic Texoma Max Q II (NE) for 56 days. The KY31 pastures were examined at the start of these studies and determined to exhibit a 98% endophyte infection rate [42]. Study 2 was conducted based on the findings of study 1. Over a two-year period, yearling AN bulls ($n = 53$; ages ≥ 12 mo with 25 and 28 bulls utilized in year 1 and 2, respectively) produced at Simpson Research and Education Center, Pendleton, South Carolina were evaluated for body weight (BW), body condition score (BCS), and scrotal circumference (SC), and allotted to two treatments consisting of either grazing toxic KY31 or NE for 56 days following a 14 day adjustment period to a forage ration. The Simpson Research and Education Center utilizes KY31 in many pastures therefore bulls in this study were previously exposed to EA. For both studies, bull BW, BCS, and SC measurements were recorded, and electroejaculation conducted at the start of TRT on day (d) 0, and every 28 days to the end of the study.

2.2. Semen evaluation

Semen was assessed as described by Stowe et al., 2013 [41] for motility, morphology, and velocity. Briefly, bulls were restrained in standard animal handling chutes and subjected to electroejaculation using the Pulsator IV (Lane Manufacturing, Inc., Denver, CO) on the preprogrammed collection mode [41]. Ejaculate volume was recorded and semen quality parameters were estimated on-site using a computerized sperm quality analyzer (SQA-Vb; A-Tech, Los Angeles, CA) specific for bull semen [48–50]. Parameters evaluated included sperm concentration, percentage of motile sperm, percentage of progressively motile sperm, motile sperm concentration, progressively motile sperm concentration, and sperm velocity. Morphology was determined by staining ~10 μl of ejaculate with Eosin/Nigrosin stain (Hancock Stain; 5% w/v nigrosin, 0.6% w/v esosin and 3% w/v sodium citrate dehydrate, pH 7.0), generating a smear on a microscope slide, and allowing the slide to air dry. Cell counts were performed using microscopic procedure with the percentage of abnormal/normal cells recorded for a minimum of 100 cells counted per sample.

2.3. PRL RIA

To assess the effectiveness of TRT in study 2, blood samples were assayed for serum PRL. Blood was collected via caudal veinapuncture, allowed to clot and placed at 4 °C overnight, and serum harvested by centrifugation at $2000 \times g$ for 15 min. Serum was placed in vials and stored at −20 °C until analyzed for PRL using RIA. Blood samples were obtained on d 0, 28, and 56. Prolactin assays were performed by the F. Neal Schrick laboratory as previously described [40].

2.4. Semen extension and artificial insemination

In studies 1 and 2, semen was collected on d 56 and evaluated as described above. Criteria for semen chosen to be extended and used in AI were based on the requirements of bulls to pass a standard breeding soundness examination [51]. All bulls utilized in studies 1 and 2 met or exceeded the SC requirements and all bulls whose semen was chosen for extension met or exceeded requirements for motility (>30%) and morphology (>70%) Semen was extended following manufacturer's directions (Andromed^{R}, Minitube USA, Delavan, WI) to a final concentration of 10×10^6 motile sperm per 0.5 mL. Semen was then loaded into 0.5 mL straws and stored at 19° C until used for timed artificial insemination (TAI) within 48 h of collection. Extended ejaculates across treatments exhibiting similar semen quality traits were chosen for TAI ($n = 2$ and $n = 4$ per treatment for studies 1 and 2, respectively). Breifly, the means for SC, percent motile sperm and percent normal sperm for semen to be extended are given in Table 1. The mean values for SC, percent motile sperm and percent normal sperm for semen extended from bulls grazing KY31 or NE in both studies 1 and 2 are above mean values for all bulls for study 1($n = 10$ bulls) and study 2 ($n = 53$ bulls).

Primi- and multiparous AN and AN-crossbred cows used for study 1 ($n = 81$; with ≥20 bred with semen from each bull) and study 2 ($n = 180$; with ≥16 cows bred to each bull) were stratified by days post-partum, BW, and BCS, and allotted to be inseminated with extended semen from bulls exposed to KY31 or NE. Cows were subjected to TAI using the 7-day CO-Synch + CIDR protocol, with all animals being inseminated beginning approximately 60 h post-CIDR removal and ending approximately 3 h after the initiation of breeding. Cows were run through the chute at random and breeding