Reproductive characteristics of endophyte-infected or novel tall fescue fed ewes

J.M. Burke a,*, C. Bishop b, F. Stormshak b

a USDA, Agricultural Research Service, Booneville, AR, 72927-9214, United States
b Department of Animal Sciences, Oregon State University, Corvallis, OR 97331-6702, United States

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Abstract

Reduced pregnancy rates often occur in ruminants grazing endophyte-infected (EI) tall fescue. The objectives were to characterize basal and oxytocin-induced PGF2α concentrations in serum and reproductive function in ewes fed tall fescue seed and to determine whether addition of fish meal (FM) to a diet of EI fescue would alter PGF2α production. Ewes were fed a diet with novel or non-toxic endophyte-infected (NE) or EI tall fescue seed containing either corn gluten meal (CG) or FM. Serum concentrations of prolactin, a measure of severity of fescue toxins, were reduced in ewes fed EI compared with NE forage seed (forage \times day, \( P < 0.02 \)) and were greater in NEFM than NECG-fed ewes (\( P < 0.03 \)). Size and number of corpora lutea (CL), determined by trans-rectal ultrasonography, were similar between diets (\( P > 0.10 \)). Serum concentrations of progesterone were reduced in ewes with two CL fed EI compared with NE seed (forage \times CL number \times day, \( P < 0.001 \)). Oxytocin-induced PGFM concentrations during the luteal phase were determined as a measure of uterine function. On the day of oxytocin administration, peak plasma concentrations of PGFM were reduced in EI compared with NE-fed ewes (forage \times time, \( P < 0.003 \)), but FM did not influence PGFM concentrations. Estrous cycle length was more variable in EI than NE-fed ewes. There appears to be some asynchrony between NE and EI-fed ewes leading to changes in uterine responsiveness to oxytocin. Inclusion of FM did not alter uterine responsiveness to oxytocin.

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1. Introduction

Grazing endophyte-infected (EI) tall fescue (Festuca arundinacea) can lead to decreased animal performance due to ergot alkaloids produced by the endophyte (Neotyphodium coenophialum) within the plant (Ball et al., 1996). Eradication of this cool season perennial grown on more than 14.2 million ha...
signs of fescue toxicosis in ruminants grazing tall fescue include decreased systemic prolactin, increased respiration rate and core body temperature and decreased feed intake (Porter and Thompson, 1992). Pregnancy and calving rates have also been reduced in heifers grazing EI tall fescue (Brown et al., 1992; Gay et al., 1988) leading to more than $300 million lost annually to the beef industry (Hoveland, 1993). Changes in reproductive responses may be associated with an asynchrony of reproductive hormones that occurs during the cycle and a change in luteal and follicular dynamics (Burke et al., 2001; Burke and Rorie, 2002). Estrous cycle length may be shortened (Jones et al., 2003). Serum concentrations of prostaglandin (PG)13,14-dihydro-15-keto F2α metabolite (PGFM), a stable metabolite of PGF2α, were increased in heifers and cows administered an acute dose of ergotamine tartrate (Browning et al., 1998, 2001), an ergopeptine alkaloid associated with Neotyphodium (Bush et al., 1979; Siegel et al., 1990). An earlier rise in PGF2α could lead to earlier regression of the corpus luteum and a shortened estrous cycle. Fish meal (FM), rich in polyunsaturated fatty acids, eicosapentaenoic and docosahexaenoic acids, fed to dairy cattle led to decreased plasma concentrations of oxytocin-induced PGFM (Thatcher et al., 1997; Mattos et al., 2002) and could reduce a fescue toxin-induced increase in PGF2α.

Recently, a novel EI (NE) tall fescue variety was developed by infecting a non-toxin-producing endophyte into an EF variety of tall fescue (Bouton et al., 2002). Jesup tall fescue was infected with a non-ergot alkaloid-producing strain that possessed greater yield and stand survival than EF fescue. Gains were 57% greater and serum concentrations of prolactin were similar in lambs grazing Jesup or EF compared with EI fescue (Bouton et al., 2002; Parish et al., 2003a). Calf average daily gain and gain/ha were also greater on NE than toxic EI fescue (Parish et al., 2003b).

The objectives of the current study were to 1) characterize basal and oxytocin-induced PGF2α concentrations in serum and luteal dynamics in ewes fed EI fescue compared with NE tall fescue and 2) determine whether addition of FM to a diet of NE or EI fescue would alter oxytocin-induced PGF2α response or alter luteal life span in ewes.

2. Materials and methods

All experimental procedures were reviewed and accepted by the Agricultural Research Service Animal Care and Use Committee in accordance with the NIH Guide for the Care and Use of Laboratory Animals. Pain and stress to animals were minimized throughout the experimental period.

2.1. Animals and treatments

In late summer, yearling Dorset ewes, raised on the Booneville Agricultural Research Service facilities, that were determined to be cycling (observed in standing estrus for vasectomized ram) and in good body condition (mean of 3.8 body condition score), weighing approximately 50 kg, were assigned randomly to be individually fed NE (‘Jesup’ variety; MaxQ, Pennington Seed, Inc., Madison, GA), which contained no ergot alkaloids associated with fescue toxicosis, or EI (‘Plantation’ variety; Pennington Seed) seed diet for an average of 52 days. The fescue diets contained either corn gluten meal (CG) or Menhaden FM (Sealac; Omega Protein, Hammond, LA) as supplemental protein sources that were balanced for undegradable intake protein (n = 5 ewes/NECG, NEFM, and EIFM; n = 4 ewes/EICG). Diets were 50% ground fescue seed, cracked corn (CG, 11.6%; FM, 15.4%), 15% cottonseed hulls, 6% molasses, 6% soybean hulls, 8% CG or 5.4% FM, 0.69% vitamin/premix, limestone (CG, 1.36%; FM, 0.98%), and 0.5% salt on a dry matter basis. For all diets, crude protein was estimated to be 14% and total digestible nutrients was 71% of the diet. Ewes were fed at 08:00 daily and weight of orts determined. Consumption of EI tall fescue is associated with reduced intake (Hemken et al., 1979). To minimize a confounding effect of reduced feed intake, feed offered to the NE group was adjusted to the average intake (as a percentage of body weight) of the EI group (fed ad libitum) from the previous day, resulting in an average dry matter
intake of 1.2 kg/day or 2.4% of body weight. NRC (1985) recommendations for crude protein and total digestible nutrients were met. Ergovaline concentration in seed was determined by high-performance liquid chromatography (Rottinghaus et al., 1991) and was 0 and 3.2 μg/g ergovaline for NE and EI fescue seed, respectively, resulting in consumption of 39.8 μg ergovaline/kg body weight in the EI ewes. A 50 kg ewe was estimated to consume 1.9 mg of ergovaline. Consumption of ergovaline from primarily EI tall fescue pasture would be between 2.2 to 132 μg ergovaline/kg body weight (Burke et al., 2001). Ewes continued the diet until observed in estrus that followed administration of oxytocin. After ewes were bred, they were fed Bermuda grass hay ad libitum and 225 g corn/soybean meal supplement without further tall fescue seed or protein (CG or FM) supplementation.

Rectal temperature was determined daily at 13:00. At that time ambient temperature was recorded and ranged between 24 and 33 °C throughout the trial. Body weight was determined on Days 0, 28, and 56 after starting diets.

Ewes consumed diets for 12 to 28 days before Day 0 (standing estrus) of the ultrasound monitored estrous cycle (Aloka SSD 500 V ultrasound scanner equipped with a 7.5 MHz linear array prostate transducer; Aloka Co. Ltd, Japan; Days 0, 2, 4, 6, 8, 10, 12, 13, 14, 15 or until subsequent estrus occurred). Size and numbers of corpora lutea (CL) were recorded. On Day 15 of the estrous cycle or at first sign of CL regression (based on ultrasonic images; 1 NEFM ewe on Day 14, 2 EICG ewes on Day 13, and 3 EIFM-fed ewes on Day 12, 13, and 14), ewes were fitted with an indwelling 16 Ga jugular catheter (Angiocath; Becton Dickinson, Sandy, UT) and oxytocin (40 IU; RX Veterinary Products, Grapevine, TX) was administered IV. Blood was collected using 10-ml vacutainer tubes, the samples placed on ice and centrifuged at 3000×g at 4 °C for 15 min. The resulting sera were stored at −20 °C until analyzed for prolactin and progesterone. Concentrations of prolactin were determined by use of a double antibody RIA procedure of Spoon and Hallford (1989). Samples were run in a single assay having a CV of 13% and a recovery of added prolactin of 97%. Serum concentrations of progesterone were determined in a single assay commercial solid-phase RIA kit (Diagnostic Products Corp., Los Angeles, CA) validated by Schneider and Hallford (1996) in multiple assays; intra- and inter-assay CV were 9.2% and 6.1%, respectively. Plasma concentrations of PGFM were analyzed directly using a polyethylene glycol RIA procedure described by Eley et al. (1981) and Guilbault et al. (1984). Intra-
and inter-assay CV were 3.9% and 6.0%, respectively. Sensitivity was 2.5 pg/ml; therefore, samples that were below the sensitivity of the assay were assigned this value.

2.3. Statistical analysis

Data collected over time were analyzed using the Mixed Models procedure of SAS (1996) with a repeated statement for time of measurement (Littell et al., 1996). The statistical model for concentration of hormones, CL diameter, body temperature, or body weight included diet (2 × 2 factorial with forage seed variety and protein source as the main effects), time or day, and the interactions. Means were separated using the PDIFF option which requests that P-values for differences of the LS-means be produced. Pregnancy rate and number of embryos were analyzed by general linear models procedures and categorical analysis or Cat Mod procedures of SAS. Fertilization rate was estimated as the proportion of ovulated follicles, measured as the number of CL, which produced live embryos. Heterogeneity of regression was used to examine effect of diet on the relationship between response variables and day of feeding. Day was tested to the order of significance.

There were two NECG, one NEFM, three EICG, and two EIFM ewes that formed a second CL. Analysis of CL diameter over day of estrous cycle was performed on the first CL that developed. For the prolactin analysis, there was a typical surge of prolactin (>200 ng/ml; Kann and Denamur, 1974) in four NE-fed ewes on day of estrus that was deleted from the analysis, as only baseline values were of interest.

3. Results

Rectal temperature was greater in EI compared with NE-fed ewes (P<0.001) and varied by day in response to change in ambient temperature (P<0.001; Fig. 1). Dietary protein had no influence on rectal temperature. Serum concentrations of prolactin were 55 ng/ml in four NE-fed ewes on day of estrus that was deleted from the analysis, as only baseline values were of interest.
lactin were increased in FM-fed ewes ($P<0.03$) and were nearly abolished in EI-fed ewes (forage $\times$ day; $P<0.02$; Fig. 2A). Prolactin levels increased within 7 days after EI dietary treatment ended, but decreased in NE-fed ewes (forage $\times$ day; $P<0.001$; Fig. 2B). This was not influenced by pregnancy as determined by including pregnancy status as a covariate for each prolactin value. Weight gains did not differ significantly during the trial (Table 1).

The CL of one EICG and two EIFM-fed ewes could be visualized by ultrasonography on Day 2 and two EICG-fed ewes by Day 4. Otherwise CL were not visualized before Day 4. There were no differences between mean diameter of CL among forage or protein treatments and no interaction. There was no difference in number of CL among dietary groups before or after the oxytocin challenge. There were three NE-fed and five EI-fed ewes that formed a second CL, which influenced mean progesterone concentrations. Serum concentrations of progesterone decreased in EI-fed ewes when number of CL was used as a covariate ($P<0.03$; Fig. 3A). In NE-fed ewes there was an increase in progesterone associated with the presence of two CL, but in EI-fed ewes there was no increase in progesterone associated with the presence of a second CL (forage $\times$ CL number $\times$ day, $P<0.001$; $R_{\text{model}}^2=0.57$; Fig. 3B). There was no effect of dietary protein on serum concentrations of progesterone.

Peak plasma concentrations of oxytocin-induced PGFM were markedly reduced in EI-fed ewes (forage $\times$ time, $P<0.003$; Fig. 4) with no effect of dietary protein. Basal levels of PGFM were similar among treatment groups. Estrous cycle length during experimental dietary treatment was similar among groups, but was more variable for the EI-fed ewes (Fig. 5). In response to breeding after the oxytocin challenge, pregnancy rate, number of embryos, number of CL, and fertilization rate did not differ

![Fig. 3. Least squares means and standard errors of serum concentrations of progesterone determined every 1 to 2 days throughout estrous cycle for ewes fed 50% novel endophyte (NE; no ergovaline; $n=10$) or endophyte-infected (EI; $n=9$) tall fescue seed with corn gluten meal (CG) or fish meal (FM) as the protein source. Values are presented for days before ovulation (A). Regression lines are presented for serum concentrations of progesterone throughout the estrous cycle of NE-fed ewes with one (open circles; $n=7$) or two CL (open squares; $n=3$) and EI-fed ewes with one (gray circles; $n=4$) or two CL (gray squares; $n=5$) with symbols representing estimated values.](image1)

![Fig. 4. Least squares means and standard errors of plasma concentrations of PGFM from -10 to 70 min after IV infusion with 40 IU oxytocin on Day 15 of the estrous cycle or on day of first sign of CL regression based on ultrasonic image in ewes fed a diet of 50% novel endophyte (NE; $n=10$; no ergovaline; open circles) or endophyte-infected (EI; $n=9$; closed squares) tall fescue seed.](image2)
significantly among treatments (Table 1). All ewes were pregnant by the following breeding cycle.

4. Discussion

In the current study, ewes exhibited typical signs of fescue toxicosis (Hemken et al., 1979; Paterson et al., 1995; Oliver, 1997; Burke et al., 2001). Rectal temperature was increased in EI-fed ewes in response to warmer ambient temperatures. Systemic prolactin was reduced in EI-fed compared with NE-fed ewes. Systemic prolactin is often decreased because the ergot alkaloids act as dopaminergic agonists (Muller-Schweinitzer and Weidmann, 1978).

There were no changes in number or diameter of the CL in the current study, which is consistent with previous reports in beef heifers (Burke et al., 2001) or cows (Burke and Rorie, 2002). Concentrations of progesterone were similar between NE and EI-fed ewes with only one CL and increased in NE-fed, but not EI-fed ewes with two CL. In general, progesterone concentrations are greater in ewes with multiple CL (Amiridis et al., 2002). Decreased progesterone synthesis could be attributed to decreased cholesterol production, a precursor to progesterone, typical in livestock grazing EI fescue (Stuedemann et al., 1985).

In particular, cholesterol may have limited progesterone secretion in EI-fed ewes that formed two CL.

Browning et al. (1998, 2001) determined that basal PGFM was increased after administration of ergotamine tartrate (an ergopeptine alkaloid associated with Neotyphodium) to heifers 2 or 10 days after induced luteolysis or to cows 15 or 16 days post-estrus. However, oxytocin-induced PGFM response was similar between saline and ergotamine-treated heifers, though oxytocin receptors may not have been present at this stage of the cycle. Menhaden fish meal contains the omega-3 fatty acids, docosahexaenoic and eicosapentaenoic acids, which can inhibit cyclooxygenase activity and, in turn, decrease PGF2α synthesis (Smith and Marnett, 1991). Basal levels of PGFM were similar between NE- and EI-fed ewes in the current study, and further, oxytocin-induced PGFM peak was reduced in EI-fed ewes. Changes in oxytocin-induced PGF2α have not been reported in EI-fed cattle during the late luteal phase. Thatcher et al. (1997) and Mattos et al. (2002) reported a decreased peak of oxytocin-induced PGFM 15 days after ovulation or administration of hCG, respectively, in response to dietary fish meal in non-lactating and lactating dairy cows, respectively. In the current study, dietary FM had no effect on plasma concentrations of PGFM. The reason that dietary fish meal resulted in decreased peak oxytocin-induced PGFM in cows but not ewes is not clear.

There was increased variation in cycle length between forage groups, which could indicate some asynchrony between groups of ewes. Jones et al. (2003) noted a decreased cycle length in beef heifers. Individual responses to fescue toxins vary as with any stressor, which contributes to increased variation to responses with the EI treatment groups.

Differences in prolactin response were apparent among treatment groups. Ewes were subjected to afternoon heat stress conditions for approximately the first 35 days of the feeding period. As ambient temperature declined, serum concentrations of prolactin declined in NE-fed ewes. Others (Hooley et al., 1979; Walker et al., 1990) observed increased prolactin in heat-stressed ewes. The reason that dietary FM increased serum prolactin compared with corn gluten meal is not clear from these data. There was a numerical advantage in FM-fed ewes for pregnancy and fertilization rates and embryo number,
perhaps associated with the increased prolactin. Greater fertility may be associated with greater prolactin production in mice and ewes (Cecim et al., 1995; Notter and Chemineau, 2001). FM included in dairy cow rations improved pregnancy or conception rate (Armstrong et al., 1990; Bruckental et al., 1989; Burke et al., 1997).

Differences in prolactin were also observed after withdrawal of the diets. Concentrations of prolactin decreased, as expected in NE-fed ewes during early pregnancy (Kann and Denamur, 1974). Concentrations of prolactin increased within 7 days after the EI seed was withdrawn. It is not understood how an increase in prolactin at this time or during early pregnancy in EI-fed ewes could lead to decreased pregnancy rates. A dual role of LH and prolactin has been suggested for CL maintenance both during the cycle and in early pregnancy (Tabarelli et al., 1982; Kann and Denamur, 1974). However, changes in luteinizing hormone were not observed in EI-fed heifers (Mizinga et al., 1992) or ewes administered an ergopeptide (Louw et al., 1974). The relevance of decreased prolactin in EI-fed animals has never been clearly demonstrated.

Novel EI tall fescue varieties have been developed to alleviate problems in livestock associated with tall fescue. Clearly, NE-fed ewes in this study lacked signs of fescue toxicosis. There were increased gains in lambs and calves grazing NE compared with EI fescue (Bouton et al., 2002; Parish et al., 2003a,b) and improvements in pregnancy rate in Angus cows (Burke et al., 2004). While fescue toxicosis does not appear in ruminants grazing these new varieties of tall fescue, replacement of EI fescue pastures can be timely and costly. In addition, there is evidence from the current research location that annual forage production may be reduced compared with EI varieties (Burke and Brauer, unpublished data). Management of ruminants should consider breeding during cooler times of the year or use of pastures with alternative forages.

5. Conclusion

Evidence of hormonal asynchrony in EI-fed ewes included altered PGF$_2\alpha$ response to oxytocin, increased prolactin in early pregnancy, and greater variation in cycle length. All these could potentially lead to changes in embryo development or loss, which is often observed in beef heifers grazing EI tall fescue. Addition of dietary fish meal did not change plasma concentrations of PGFM, but increased prolactin in cycling ewes.

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References


Siegel, M.R., Tapper, B.A., Bacon, C.W., Johnson, M.C., 1990. Alkaloids and