Evaluation of Domperidone Dosages and Delivery Methods for the Treatment of Fescue Toxicosis in Beef Heifers


*Department of Animal Science, Food and Nutrition, Southern Illinois University Carbondale, Carbondale, IL; †USDA-ARS, Forage-Animal Production Research Unit, Lexington, KY; ‡Equitox, Clemson, SC; §Burns Biosolutions, Lexington, KY; ¶BioRelease Technologies, Birmingham, AL; ||St. Joseph’s Health Centre, London, Ontario, Canada; ¶Department of Veterinary Medicine, University of Illinois Urbana-Champaign, Champaign, IL; and **School of Animal Sciences, Louisiana State University Agricultural Center, Baton Rouge, LA

ABSTRACT

The objective of this study was to develop a practical method of domperidone delivery to ameliorate fescue toxicosis. Experiment 1 used heifers assigned to 7 treatment groups (n = 6 each): positive control (0.44 mg domperidone/kg BW daily s.c. for 9 d), negative control, and 0.22, 0.44, 0.88, and 1.76 mg domperidone/kg BW per os daily for 9 d, or a 3 g domperidone i.m. injection. Blood was collected every third day for 24 d. Domperidone concentrations in the 0.88 and 1.76 mg/kg BW treatments and the i.m. treatment were greater than positive control (P < 0.05) on d 3. None of the oral treatments were greater than the positive control on subsequent days. Between d 6 and 24, no oral treatments differed from the negative control except for the 1.76 mg/kg of BW treatment on d 9. The i.m. formulation increased domperidone when compared with the negative and positive controls (P < 0.05) on d 3 through 21. Experiment 2 evaluated the i.m. injection protocol on performance. Heifers were assigned to control (n = 15) or i.m. domperidone (n = 15) treatments and grazed endophyte-infected fescue paddocks. Blood was sampled weekly and analyzed for progesterone and prolactin concentrations. Controls had reduced BW gains (P < 0.001) and BCS (P < 0.05) and elevated rectal temperatures (P < 0.05) compared with treated heifers. Domperidone treatment interacted with day on affecting prolactin (P < 0.0001) and progesterone (P < 0.0001). Intramuscular delivery of domperidone is an effective method for relieving fescue toxicosis.

Key words: bovidae, antagonist, prolactin, progesterone, body weight

INTRODUCTION

Consumption of toxic endophyte-infected tall fescue [Lolium arundinaceum (Schreb.)] by livestock is associated with a condition termed fescue toxicosis. Ergot alkaloids produced by the endophyte are thought to be the primary cause of fescue toxicosis (Porter, 1995). In cattle, the condition reduces productivity by decreasing weight gains, reproductive efficiency, and milk production. These compounds reduce prolactin, increase body temperatures (Porter and Thompson, 1992), and increase vasoconstriction (Solomons et al., 1989). As a result of poor animal performance, the beef industry experiences losses of more than $600 million annually (Paterson et al., 1995). Domperidone (Equitox, Clemson, SC) therapy has proven to be an effective method to counteract negative responses to infected-fescue consumption (Redmond et al., 1994; Jones et al., 2003, 2004). In these
studies, subcutaneous domperidone, a dopamine antagonist, ameliorated some of the deleterious reproductive effects, reduced weight gains, and altered gene expression profiles associated with fescue toxicosis in cattle. Other routes of administration to cattle have not been thoroughly explored and would need to be less labor intensive for producers. The following experiments evaluated the effects of oral and slow release domperidone administration on fescue toxicity symptoms in cattle. In Exp. 1, we hypothesized that domperidone administered orally or by slow-release injection would achieve circulating domperidone levels similar to subcutaneous administration. In Exp. 2, we hypothesized that the slow-release administration of domperidone would improve performance as compared with the non-treated controls.

**MATERIALS AND METHODS**

All animal procedures were approved by Southern Illinois University Carbondale’s Animal Care and Use Committee (04-040 and 05-016).

**Experiment 1**

Forty-two crossbred Angus heifers (BW 320 ± 6 kg) 18 to 24 mo of age were blocked by calving season (fall or spring calf) and BW. Six heifers were assigned to each of 7 treatment groups. The study was conducted during December 2004 and January 2005 at the Dixon Springs Agricultural Center, Simpson, IL. All heifers were fed free-choice clover-bromegrass hay, free-choice commercial mineral (Renaissance Nutrition, Roaring Spring, PA), and a grain ration containing 85% corn and 15% soybean meal, with ad libitum access to water. Heifers in treatment groups 1 through 6 were housed by treatment at one location in pens of similar size ranging from 16.0 m × 3.9 m to 16.0 m × 4.5 m, all with sheltered areas. Due to insufficient pen numbers, heifers in treatment group 7 were housed at an additional facility approximately 1.6 km away in a 12.9 m × 14.7 m pen with a sheltered area.

Treatment group 1 (positive control) received a daily injection of 0.44 mg domperidone/kg BW s.c. for 9 consecutive days (0 to 8 d), a treatment regime similar to that of Jones et al. (2003). Group 2 (negative control) received no domperidone or placebo. Heifers in groups 3 through 6 received oral administration of approximately 0.22, 0.44, 0.88, or 1.76 mg domperidone/kg BW daily for 9 consecutive days (0 to 8 d; Figure 1). Domperidone was administered as an oral paste (11% wt/vol; Equitox) and dosage was rounded to the nearest 0.5 mL. Actual dosages were 0.17 ± 0.01, 0.43 ± 0.02, 0.94 ± 0.01, or 1.72 ± 0.03 mg/kg BW for the respective oral treatment groups. Heifers in group 7 received an i.m. slow-release domperidone (3 g) microparticle (Poly D,L-lactide-co-glycolide) formulation as provided by BioRelease Technologies (Birmingham, AL) once on d 1. The suspension vehicle contained carboxymethylcellulose as a thickening agent to suspend the domperidone microparticles. This is the same dosage and delivery method recently described for use in mares (Kelley et al., 2006). The domperidone depot (20 mL) was delivered by 16 gauge × 1 1/2" needles to the neck. The total depot was delivered in 2 to 4 injection sites depending upon whether the needle clogged.

Blood samples (10 mL) were collected via tail vein or jugular venipuncture 6 d before treatment and every third day during the treatment and withdrawal periods. Arrow on d 1 indicates the timing of the i.m. domperidone injection.

Samples were centrifuged at 655 × g for 10 min. Serum samples were frozen at —20°C until analyzed. To determine the oral absorption pattern of domperidone, serial blood samples were collected from heifers in group 6 on d 9. Blood was collected at 0, 2, and 4 h after drug administration. Samples were harvested and stored as described previously. All samples were shipped overnight with dry ice to St. Joseph’s Health Centre, London, Ontario, Canada. Samples were analyzed for domperidone concentrations by HPLC—electrospray mass spectrometry using established methods (Zavitsanos et al., 1999). Domperidone standards were prepared in bovine albumin at the following concentrations: 0, 0.5, 1.0, 2.0, 4.0, 8.0, 10.0, and 20.0 ng/mL. The internal standard was R68808. The extraction solvent was composed of 49.5% isooctane (HPLC grade; J.T. Baker, Phillipsburg, NJ), 49.5% dichloromethane (VWR-Canlab, Mississauga, Ontario, Canada), and 1% isoamyl alcohol (Fisher Scientific, Toronto, Canada).
The experiment was conducted on an HP 1100 LC-diode array detection-mass spectrometry system (Hewlett-Packard, Palo Alto, CA). The ion for domperidone was m/z 426.1 and that for the internal standard was m/z 421.3. After acquisition, the resultant chromatograms were integrated by the Hewlett-Packard Chemstation software. The integration parameters were determined on low-level standards and were subsequently used without modification. Baselines were set automatically and were not manually altered. The limit of detection for domperidone was 0.25 µg/L serum and the limit of quantitation was 0.5 µg/L serum.

**Experiment 2**

The trial took place from July to October 2005 on the Southern Illinois University Carbondale farms. Thirty Angus or Simmental cross-bred heifers (average BW 344.5 ± 7.4 kg) 18 mo of age and with an average BCS of 5.4 ± 0.6 were purchased from local sources.

Heifers were sorted based on BW and BCS and equally allotted into 2 groups. Treatments were either control, with heifers receiving no treatment or carrier, or treatment group, in which each heifer received i.m. domperidone as described in Exp. 1 (Figure 2). The 14.5-ha pasture was at one location, unimproved, and included fescue as well as other grass types. Heifers were adapted to this pasture for 1 mo before the study. Heifer dietary history previous to this time was unknown. All paddocks were sampled at the initiation of the trial in July. Grass samples were pooled (n = 5 samples/paddock) and submitted for ergot alkaloid concentrations (G. Rottinghaus, University of Missouri Veterinary Diagnostic Lab., Columbia, MO). The ergovaline composition of the grass was 160 ppm of ergovaline, with no ergot (Claviceps spp.) present. Further testing of the pasture was not performed even though ergovaline concentrations may fluctuate over time and season (Tor-Agbidye et al., 2001).

Heifers grazed as one group and rotated between four 2.8- to 4.0-ha paddocks every 5 to 7 d. Cattle were supplemented with 0.9 kg cracked-corn/heifer daily and had ad libitum access to water.

Estrous cycles were synchronized using 2 prostaglandin injections (25 mg i.m., Lutalyse; Pharmacia and Upjohn, Kalamazoo, MI), 11 d apart (d −10 and 0 of the study). One heifer in the control group aborted and was removed from the study. Estrous cycle synchronization was timed such that the second prostaglandin injection was given the same day as the domperidone injection (Figure 2). All heifers were fitted with Kamar Heatmount Detectors (Kamar Inc., Steamboat Springs, CO) at time of second prostaglandin injection to aid in visual detection of estrus. Daily transrectal ultrasonography was performed (Aloka SSD-500V; ALOKA Ultrasound, Wallingford, CT) on d 1 to 4 to determine follicle size and time of ovulation. Artificial insemination was performed by experienced technicians via a double insemination protocol using straws from the same lot of semen. The first artificial insemination was performed 72 h after the second prostaglandin injection, or before 72 h if the Kamar was activated and if a follicle greater than 10 mm was observed by ultrasound; or if the animal was observed in standing heat, whichever occurred first. The second artificial insemination occurred approximately 24 h after the first insemination. Blood was drawn 42 d postinsemination to determine pregnancy using BioPRYN Pregnancy Test (BioTracking LLC, Moscow, ID).

Blood was collected via jugular venipuncture at weekly intervals (d −3, 4, 11, 18, and 25) to determine circulating prolactin concentrations and on d −3, 4, 11, 14, 18, and 25 to determine circulating progesterone concentrations. Samples were immediately transferred to heparinized vaccutainer tubes and stored on ice until processed. All blood samples were centrifuged (IEC Centra GP8R, Therm Scientific, Milford, MA) at 655 × g for 10 min within 3 h of collection. After centrifugation, all samples were aliquoted and stored in a −20°C freezer until analyzed. Progesterone concentrations were determined by RIA (Coat-A-Count; Diagnostic Products, Los Angeles, CA). Limit of quantitation was 0.03 ng/mL. Intra- and interassay CV were 2.2 and 3.7%, respectively. Plasma prolactin concentrations were determined by an RIA similar to that used by Miller et al. (1999). This RIA, which we validated for cattle tissues, was based on an ovine prolactin antiserum (AFP-C358106) and had a sensitivity to 0.25 ng/mL plasma. Intra- and interassay CV were 6 and 11%, respectively.

Body condition score and BW were recorded approximately 7 d before domperidone administration and 25 d post-administration. Body condition scores were measured on a scale of 1 to 9, with 1 being ex-
tremely underweight and 9 being obese (Richards et al., 1986). Body condition scores were evaluated by 3 individuals and the consensus score was used for statistical analysis. The evaluators were not blinded to treatment group. Rectal temperatures were measured using a rectal thermometer (M 216; GLA Agricultural Electronics, San Luis Obispo, CA) between 0700 and 0900 h each time the animals were handled for the other procedures. Daily ambient temperature and humidity were recorded at 1400 h using a hand-held digital thermometer (Control Co., Friendswood, TX) at the pasture site.

Statistical analyses

Statistical analyses were performed using SAS (version 9.1; SAS Inst. Inc., Cary, NC) or SPSS (Version 11.5; SAS, Chicago, IL). Domperidone, hormone concentrations, and body temperatures were analyzed using the PROC MIXED procedures of SAS with day as the repeated measure. Post-hoc testing of treatment by day differences was performed using PDIFF. Differences in BW, differences between initial and final BCS, and largest follicle size were analyzed by one-way ANOVA using SPSS with treatment as the independent variable. Effects were considered significant at $P < 0.05$.

**RESULTS AND DISCUSSION**

**Experiment 1**

Treatment, day, and treatment by day were all statistically significant ($P < 0.001$) in the overall model. Comparing treatments within each day revealed that heifers receiving 0.88 or 1.76 mg/kg BW oral domperidone treatment showed a significant increase ($P < 0.05$) of circulating domperidone on d 3 (Table 1). Results from the serial blood samples collected on d 9 showed the highest concentration of domperidone (5.57 ± 0.48 µg domperidone/L serum) occurred at 2 h after administration of the oral paste, and concentrations decreased to $1.07 ± 0.16$ µg domperidone/L serum by 4 h after administration.

No differences in domperidone concentration were detected between all oral doses and the negative control after d 3 ($P > 0.05$) except for the 1.76 mg/kg BW treatment on d 9 ($P < 0.05$). Heifers receiving the daily s.c. injection of 0.44 mg domperidone/kg BW showed circulating concentrations between 1.2 and 2.9 µg/L serum, which were highest at d 12 of the experimental period. Heifers receiving the i.m. injection of domperidone had greater circulating concentrations ($P < 0.05$) than negative and positive control groups for d 3 through 21. Circulating levels were highest on d 12 for the positive control (2.9 µg domperidone/L serum) and for the i.m. treatment (10 µg domperidone/L serum), and then decreased for the remainder of the experiment to non-quantifiable or near non-quantifiable levels by d 24 (Table 1).

**Experiment 2**

Beginning (5.5 ± 0.2 control, 5.4 ± 0.2 i.m.) and ending (5.0 ± 0.2 control, 5.2 ± 0.1 i.m.) BCS tended ($P = 0.06$) to be different. Body condition scores of heifers in both treatment groups declined during the study; however, the absolute magnitude of loss was greater in the untreated group. Body weight gains were increased in heifers receiving the domperidone treatment ($P < 0.001$) with mean BW increases of 4.9 ± 1.7 kg for control heifers and 13.3 ± 1.6 kg for treated heifers.

Treatment interacted with day in affecting ($P < 0.0001$) rectal temperature (Figure 3), Body temperature were elevated in the control heifers after d 4 as compared with the domperidone-treated heifers ($P < 0.05$). Daily ambient temperatures ranged from 18 to 36°C with daily humidity ranging from 61 to 96%.

Further examination of our data obtained from the progesterone RIA analysis, ultrasound data, and observations made with the Kamar heat detector suggested 7 control heifers and 9 domperidone-treated heifers were not cycling as predicted by the synchronization protocol or were not reproductively sound and were there-
Treatment by day interacted and domperidone-treated heifers. Concentrations (ng/mL serum) in control heifers (n = 15 per treatment). Treatment by day interacted (P < 0.0001).

Before excluded from further analyses. Treatment interacted with day on affecting (P < 0.0001) progesterone concentration (Figure 5). Average plasma progesterone concentrations in domperidone-treated heifers were greater than control heifers (Figure 5). Follicle size in control heifers (12.7 ± 0.3 mm) did not differ (P = 0.14) compared with treated animals (11.5 ± 0.8 mm). Conception rate of control heifers (n = 8) was 0% and in domperidone-treated heifers (n = 6) was 33%.

The results of this study indicate that a slow-release injection is a plausible method of domperidone delivery. Of concern was that the negative control group in Exp. 1 had quantifiable levels of domperidone on d 3 to 12. This observation is likely due to automated integration. Greater accuracy would likely have been obtained if manual integration had been performed. This assay was developed for use in human serum and breast milk samples, in which expected concentrations would be between 0.5 and 10.0 µg/L (Zavitsanos et al., 1999). Our samples resulted in concentrations that were at the lower limits of quantitation for this assay. Although domperidone concentrations were initially elevated (between d –6 and d 3) in the oral administration treatment groups, sustained domperidone concentrations were not observed. Possible reasons for this may be due to ruminal degradation of the compound, failure of the compound to cross from the digestive tract to the blood circulation, changes in clearance dynamics, or from absorption followed by near complete catabolism within 24 h of administration. Our serial blood samples on d 9 indicated a rise in domperidone concentration within 2 h of administration. Our serial blood samples on d 9 indicated a rise in domperidone concentration within 2 h of administration that declined at 4 h. Thus, domperidone can enter the bloodstream from the digestive tract; however, the kinetics in cattle are unknown. These results suggest that blood sampling concurrent with drug administration may not have been optimum for detecting peak circulating domperidone concentrations. Further investigations would need to be conducted to determine the metabolic fate of domperidone.

For several reasons we chose to continue the second experiment using the slow-release injection. First, the results from Exp. 1 indicated that sustained circulating levels of domperidone were possible with the slow-release injection. Second, the objective of this experiment was to identify a method of delivery practical for producers. With these objectives in mind, the one-time injection was more feasible than a daily administered paste.

Circulating prolactin is often decreased because the ergot alkaloids found in endophyte-infected fescue act as dopaminergic agonists at the pituitary (Muller-Schweinitzer and Weidmann, 1978; Strickland et al., 1992). Previous research indicated that administration of dopamine antagonists to cattle (Lipham et al., 1992; Samford-Grigsby et al., 1997) or to horses (Cross et al., 1995) fed endophyte-infected fescue increased prolactin concentrations. Results obtained from the present study demonstrated that prolactin levels were elevated in heifers receiving the dopamine antagonist, domperidone. Prolactin concentration in control heifers continued to decline throughout the study.

Previous research has shown that the consumption of endophyte-infected fescue alters progesterone levels in mares (Brendemuehl et al., 1994) and heifers (Jones et al., 2003). A decline in circulating progesterone associated with fescue toxicosis has been reported in pregnant horses (Monroe et al., 1988) and nonpregnant heifers (Mahmood et al., 1994; Burke et al., 2001). An increase in serum progesterone levels in domperidone-treated beef cows grazing endophyte-infected pastures was reported by Campbell et al. (1999) and Jones et al. (2004). In the current study, initial progesterone levels

![Figure 3](image_url) Average ± SEM rectal temperatures (°C) of control and domperidone-treated heifers (n = 15 per treatment). Treatment by day interacted (P < 0.0001).

![Figure 4](image_url) Mean ± SEM prolactin concentrations (ng/mL serum) in control and domperidone-treated heifers. Treatment by day interacted (P < 0.0001).

![Figure 5](image_url) Mean ± SEM progesterone concentrations (ng/mL plasma) in control and domperidone-treated heifers. Treatment interacted with day (P < 0.0001).
did not differ ($P > 0.5$) on d 14 of the study (midcycle) between control and treated heifers. However, upon further analysis of heifers considered to be cycling normally, progesterone concentrations were reduced in the untreated (control) group. Because progesterone is the pregnancy maintenance hormone, reduced concentrations may result in failure to maintain an otherwise viable pregnancy. This is supported by our observation that none of the control heifers were pregnant at d 42 whereas 2 of 15 (2 of 6 reproducibly sound) of the domperidone-treated heifers were pregnant at the same time period.

Consumption of endophyte-infected tall fescue diets has increased rectal temperature by 0.4 to 1.2°C (Hemken et al., 1979; Schmidt et al., 1982; Hannah et al., 1990). According to research conducted by Aldrich et al. (1993), when steers were fed endophyte-infected diets, core body temperature was 0.9°C higher in steers on endophyte-infected than endophyte-free diet; however, with supplementation of metaclopramide, a dopamine antagonist, rectal and skin temperatures were unaffected by treatment. Treatment with daily s.c. injections of domperidone has been shown to mediate body temperature increases (Jones et al., 2003). In the present study, body temperatures agreed with previous reports. Treated heifers had lower average rectal temperatures than untreated control animals.

Heifers receiving domperidone had markedly increased BW gains when compared with heifers in the control group. This result is similar to research conducted by Jones et al. (2003) in which heifers limit-fed endophyte-free or endophyte-infected diets and administered domperidone did not differ in weight gain, but those limit-fed endophyte-infected diets without domperidone treatment had depressed BW gains. Reduced BW gains in endophyte-infected fed heifers may be attributable to reduced gut motility caused by dopamine-mimicking compounds such as ergovaline, found in endophyte-infected fescue (Sorraing et al., 1984). Heifers receiving domperidone may have gut motility restored due to the dopamine blocking action of domperidone, allowing for greater weight gains when grazing endophyte-infected fescue. In this study, BCS were affected by administration of domperidone with domperidone-treated heifers losing less body condition and gaining more BW than those in the untreated group.

After evaluating ultrasound, progesterone, and Kamar Heatmount Detector data, it was determined that only 8 heifers in the control group and 6 of the treated heifers were reproducively sound and ovulated in accordance with the synchronization protocol. Reproductive status did not appear to affect plasma prolactin in either group, suggesting that the pituitary responded similarly in all heifers studied. Follicle size in the present study was similar to those observed by Jones et al. (2003), with domperidone-treated heifers having numerically smaller graffian follicular diameters than untreated heifers. Reduction in follicle size can lead to reduced luteal size and decreased progesterone concentrations (Vasconcelos et al., 2001). Low circulating progesterone increases luteinizing hormone pulse frequency (Roberson et al., 1989), leading to impaired embryo quality (Ahmad et al., 1995). The authors chose a prostaglandin-only protocol for estrus synchronization over protocols using progesterone so that progesterone data and oocyte (via fertilization rate) quality data were not compromised. Reduced progesterone concentration has been shown to reduce oocyte quality (Taft, 1999). The lack of success in synchronizing the heifers may indicate that heifers grazing endophyte-infected fescue could potentially be less responsive to prostaglandin. All heifers in this study had progesterone levels of at least 8.4 ng/mL plasma on either d −3 d or 4. This suggests the heifers were cycling at the initiation of the experiment and should have been responsive to prostaglandin administration. As reported previously, the corpus luteum gene expression profile differs (Jones et al., 2004), and prostaglandin secretion is increased in fescue-fed heifers (Browning et al., 1998). Other commercial synchronization protocols may be more successful in synchronizing heifers fed endophytic fescue in a commercial setting.

**IMPLICATIONS**

Results of this study indicate that a slow-release injection of domperidone is an effective method of domperidone delivery for relieving the symptoms of fescue toxicosis as evidence by amelioration of elevated body temperatures, reduced BCS, reduced prolactin levels and reduced progesterone levels. At this time, domperidone has not been approved for use in food producing animals. Veterinarians and producers should not use domperidone in an off-label manner. Our results suggest altering the slow-release injection composition to deliver a constant dosing of domperidone for a 60-d breeding season should be investigated. This would allow producers to easily incorporate this method into their production practices. Further investigations need to be conducted to determine the economic feasibility of domperidone usage. In addition, research evaluating the digestive fate of domperidone should be assessed for continued development of oral domperidone feeding strategies.

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