

Rumen-protected arginine alters blood flow parameters and luteinizing hormone concentration in cyclic beef cows consuming toxic endophyte-infected tall fescue seed

M. A. Green,* B. K. Whitlock,† J. L. Edwards,* E. J. Scholljegerdes,‡ and J. T. Mulliniks*¹

*Department of Animal Science, University of Tennessee, Knoxville 37996; †Department of Large Animal Clinical Sciences, University of Tennessee, Knoxville 37996; and ‡Department of Animal and Range Sciences, New Mexico State University, Las Cruces 88003

ABSTRACT: The objective of this study was to determine the effect of rumen-protected arginine on median caudal artery blood flow and LH dynamics in cows fed toxic endophyte-infected tall fescue seed. Four ruminally cannulated nonlactating beef cows (539 ± 30 kg) were used in a 2×2 factorial arrangement of treatments utilizing a 4×4 Latin square design with 4 periods of 31 d each. Each cow was assigned to individual pens and fed orchardgrass hay (10.3% CP and 85% NDF; OM basis) during a 10-d adaptation period, followed by a 21-d collection period in which each cow was assigned 1 of 4 treatments: 1) rumen-protected Arg (180 mg/kg of BW) and 1.0 kg/d of toxic endophyte-infected fescue seed (AE+), 2) rumen-protected Arg (180 mg/kg of BW) and 1.0 kg/d of endophyte-free fescue seed (AE-), 3) 1.0 kg/d of toxic endophyte-infected fescue seed (E+) alone, or 4) 1.0 kg/d of endophyte-free fescue seed (E-) alone. In each period, Doppler ultrasound measurements for blood flow parameters were quantified on d 1, 5, 10, 15, and 20. On d 20 of each period, blood samples were collected every 10 min for 6 h and then once every hour for 12 h for LH response following exogenous GnRH.

There was an Arg \times fescue seed type interaction ($P = 0.05$) for median caudal artery blood flow due to an increase in blood flow in cows fed rumen-protected Arg with endophyte-free fescue seed. In addition, mean blood flow velocity in the artery was greater ($P = 0.01$) with the inclusion of rumen-protected Arg in the diet. Median caudal artery area ($P = 0.03$) and diameter ($P = 0.01$) were decreased in cows consuming E+ compared to those consuming E- with no effect ($P \geq 0.38$) by Arg inclusion. Circulating nitric oxide (NO) concentrations tended to be influenced ($P = 0.09$) by the interaction of Arg \times fescue seed type with E+ alone decreasing NO concentrations. Circulating NO concentrations were unaffected by rumen-protected Arg ($P = 0.48$). Mean serum LH concentration exhibited ($P = 0.02$) an Arg \times fescue seed type interaction. Cows consuming E+ had decreased ($P < 0.05$) LH concentrations compared to all other treatments. However, cows consuming AE+ had ($P \geq 0.67$) LH concentrations similar to those of cows consuming AE- and E-. Thus, supplementing rumen-protected Arg to cows consuming toxic endophyte-infected fescue seed has the potential to increase reproductive performance and peripheral blood flow.

Key words: arginine supplementation, beef cows, toxic endophyte-infected fescue seed, median caudal artery blood flow

© 2017 American Society of Animal Science. All rights reserved.

J. Anim. Sci. 2017.95:1537–1544
doi:10.2527/jas2016.1309

INTRODUCTION

Tall fescue (*Schedonorus arundinaceus*), a cool-season perennial grass that grows predominately in the temperate southeast United States (Thompson et al., 2001), is grazed by over 8.5 million cattle (Hoveland,

1993). However, grazing toxic endophyte-infected tall fescue can negatively impact production in beef cattle because of the endophytic fungus *Epichloë coenophiala*, which infects 90% of tall fescue pastures (Sleper and West, 1996). A number of studies have shown the negative impact toxic endophyte-infected fescue has on pregnancy rates (Gay et al., 1988; Peters et al., 1992; Coblenz et al., 2006), follicular development (McKenzie and Erickson, 1991; Rhodes et al., 1995; Bossis et al., 1999), and embryo quality (Schuenemann

¹Corresponding author: jmulli@utk.edu

Received December 13, 2016.

Accepted February 22, 2017.

et al., 2005). In addition, systemic (Aiken et al., 2007) and reproductive (Dyer, 1993) vasoconstriction that occurs because of consumption of toxic endophyte-infected tall fescue may lead to decreased nutrient and hormone uptake in target tissues. In total, these results have been estimated to cost the U.S. cattle industry over \$600 million dollars annually (Hoveland, 1993).

Arginine (Arg) supplementation may have the potential to be beneficial in animals grazing toxic endophyte-infected tall fescue because of its role in nitric oxide (NO) and glutamate production (Wu and Morris, 1998). Nitric oxide increases blood flow by causing vasodilation and stimulating the development of blood vessels (Wu and Morris, 1998). On the other hand, Arg is a precursor for the excitatory AA, glutamate, which promotes GnRH secretion (Donoso et al., 1990). Therefore, the objective of this study is to determine the effect of supplementing rumen-protected Arg with toxic endophyte-infected or endophyte-free tall fescue seeds on peripheral blood flow, circulating LH, and serum metabolites in beef cows. The hypothesis for this study is that rumen-protected Arg will increase LH release and increase peripheral blood flow in cows consuming toxic endophyte-infected fescue seed.

MATERIALS AND METHODS

All animal handling and experimental procedures were in accordance with guidelines set by the University of Tennessee Institutional Animal Care and Use Committee. This experiment was conducted at the Joe Johnson Animal Research and Teaching Unit (JARTU) at the University of Tennessee in Knoxville (35°56'N, 83°56'W).

Animals and Treatments

Prior to the initiation of the study, 8 ruminally cannulated Angus and Angus-crossed beef cows were provided ad libitum access to orchardgrass hay (*Dactylis glomerata*) for 65 d to wash out any potential carryover effects to grazing toxic endophyte-infected fescue pastures. Cows were then brought to JARTU on d -9, which began the first day of the 10-d (d -9 to 0) adaptation period. At that time, cows were estrus synchronized utilizing the 7 d CO-Synch + controlled internal drug-releasing device (Eazi-Breed CIDR, Pfizer Animal Health, New York, NY) synchronization protocol. On d -9, each cow received a 2-mL i.m. injection of GnRH (Cystorelin, Merial, Iselin, NJ) and a CIDR inserted in the vagina. On d -2, the CIDR was removed, and the cows each received a single 5-mL i.m. injection of PGF2 α (Lutalyse, Pfizer Animal Health, New York, NY). On d 0 cows each received another 2-mL i.m. injection of GnRH (Cystorelin, Merial) to stimulate ovulation. On d 0 and 1, transrectal

ultrasonography was used by a trained technician to select the 4 cows that were most responsive to the estrus synchronization protocol. Immediately after ultrasonography, treatments were randomly assigned to the 4 cows that possessed the largest dominant follicles, whereas the remaining 4 cows were discontinued from the study and returned to the experiment station.

The 4 selected ruminally cannulated, nonlactating, cyclic cows (approximately 2.5 yr of age; 539 \pm 30 kg) were used in a 2 \times 2 factorial arrangement of treatments utilizing a 4 \times 4 Latin square design with 4 periods of 31 d each. Cows received a basal diet of orchardgrass hay (10.3% CP and 85% NDF; OM basis) fed ad libitum during the adaptation (d -9 to 0) and collection (d 1 to 21) periods at 0700 and 1700 h each day. During each 21-d treatment period, cows were assigned 1 of the 4 treatments: 1) rumen-protected Arg (180 mg/kg BW) and toxic endophyte-infected fescue seed, 2) rumen-protected Arg (180 mg/kg BW) and endophyte-free fescue seed, 3) toxic endophyte-infected fescue seed alone, or 4) endophyte-free fescue seed alone. A level of 180 mg/kg BW of arginine has been shown to improve vascular hemodynamics in steers (Meyer et al., 2011) and sheep (Saevre et al., 2011). Treatments were given intraruminally to each cow to reduce variation in supplement intake. Toxic endophyte-infected (15.5% CP and 59% NDF; OM basis) and noninfected (15.9% CP and 59% NDF; OM basis) tall fescue seeds were fed at 0.5 kg of ground seed twice daily. Toxic endophyte-infected seed was purchased from Turner Seed Inc. (Winchester, KY; Falcon IV: 2.24 mg/kg ergovaline, 1.71 mg/kg ergovalinine) and the endophyte-free seed was purchased from Tennessee Farmer's Cooperative (Monticello, KY; Kentucky 32: 0.02 mg/kg ergovaline, 0.00 mg/kg ergovalinine). Cows were housed in individual pens (2.4 \times 4.8 m) at JARTU with automatic individual waterers. A 42-d washout period occurred between treatment periods in which cows grazed eastern gamagrass (*Tripsacum dactyloides*) pastures to eliminate any potential carryover effect of the tall fescue treatments. The 42-d washout period was selected because of a minimum of 4 to 5 wk required to obtain adequate recovery from ergot alkaloid exposure (Bussard, 2012).

Tall fescue seed samples were assayed for ergovaline and ergovalinine by HPLC fluorescence using a modification of a procedure established by Yates and Powell (1988). Tall fescue seed treatments were ground through a 2-mm screen in a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA) before being dosed ruminally. Treatments containing Arg were hand mixed into the ground tall fescue seed before administration into the rumen. Rumen-protected arginine was prepared at New Mexico State University and had a rumen protection proportion of 56% (Miller, 2012).

Sampling

Color Doppler ultrasonography was used on d 1, 5, 10, 15, and 20. Each scanning period began at approximately 1400 h and ended at approximately 1600 h. Color Doppler scans of the median caudal artery under the fourth coccygeal vertebra (Aiken et al., 2007, 2009) of the tail were analyzed using a GE Healthcare LOGIQ e Vet (GE Healthcare, Chicago, IL) with a 12L-RS linear array transducer set to 2.5-cm depth. To determine the mean artery lumen area, 5 cross-sectional scans were taken for each cow, using brightness mode (B-Mode). Each individual scan was frozen during maximal flow or peak systolic phase, and then frames were saved within the memory of the ultrasound. The median caudal artery diameter 1 (cm), diameter 2 (cm), area (cm²), and circumference (cm) were traced utilizing the ellipse method. Brightness mode pulse frequency was set at 8 MHz, and gain was set at 66. Five color Doppler images were also scanned in a longitudinal transducer orientation. Color Doppler pulse frequency was set at 5 MHz, and gain was set at 13.5. Doppler insonation angle was set to 60° to retrieve a good color signal. Peak systolic velocity, end diastolic velocity, mean velocity, resistance index, and pulsatility index were measured over a cardiac cycle within each of the 5 scans and then averaged. Pulsatility index and resistance index were both used as measures of resistance within the blood vessel (Petersen et al., 1997). Throughout the experiment, all measurements were taken by the same trained technician.

On d 7, a CIDR was reinserted in the vagina of each cow to manipulate the estrous cycle to provide a sustained source of progesterone. A 5-mL i.m. injection of PGF2 α (Lutalyse, Pfizer Animal Health) was administered on d 7 and 8 to ensure regression of any corpus luteum present on the ovaries, and the CIDR was removed on d 19. On d 20 an intravenous catheter was inserted in the jugular vein of each cow 1 h prior to intensive blood sampling. A 12-gauge hypodermic needle (Ideal Instruments, Schiller Park, IL) was used to puncture the jugular vein. Approximately 0.45 m of Tygon tubing (0.10 cm i.d., 0.18 cm o.d.; Cole-Parmer Instrument Company, Vernon Hills, IL) was introduced through the needle and into the jugular vein. The remaining tubing was secured with adhesive tape to the neck of the cow and down the middle of the back. A blunt-end 18-gauge needle (Salvin Dental Specialties, Charlotte, NC) was inserted into the end of the catheter, and a 10-mL syringe was used as a tubing end cap. Eighteen hours after removal of CIDR, 9-mL blood samples were collected every 10 min for 6 h and then once every hour for 12 h. For the intensive bleeding period, cows were housed in individual bleeding stanchions (0.91 \times 2.4 m). Catheters were flushed with 5 mL of a 0.9% sterile saline immediately before and after each collection time. After collec-

tion, 9-mL blood samples were placed in Corvac serum separator tubes (Corvac, Sherwood Medical, St. Louis, MO). Blood samples were cooled and subsequently centrifuged for 20 min at 2,000 \times g at 4°C. Serum was collected and stored at -20°C in plastic vials for later analysis. Serum samples were assayed for LH concentrations by RIA using the procedures of Moura and Erickson (1997). Inter- and intra-assay CV were less than 13%, and sensitivity of the assay was 31.3 pg/mL. The number of LH pulses during the 18-h blood sampling period was determined by taking the overall LH mean concentration and then adding 1 SD. Any value greater than the LH mean plus SD was considered a pulse. In addition, LH amplitude was determined by taking the concentration of LH from the baseline to the top of the peak (Goodman and Karsch, 1980). Blood samples collected at 0 (prior to supplemental treatments), 0.5 (postsupplemental treatments), 1, 2, 4, 6, 8, 10, and 12 h were also utilized to analyze circulating NO (Cayman Chemical Company, Ann Arbor, MI) using a commercial kit.

Statistical Analysis

The normality of data distribution was evaluated using the PROC UNIVARIATE procedure (SAS Inst. Inc., Cary, NC), and all data were found to be normally distributed. Circulating blood metabolite concentrations and peripheral blood flow measurements were analyzed with sample time as the repeated factor and cow (period) as the subject with compound symmetry as the covariance structure using the MIXED procedures of SAS. The compound symmetry covariance structure was determined to be the most desirable covariance structure according to Akaike's information criterion. The fixed-effect model included treatment, sample time, treatment \times time interaction, and period as an independent variable. Random effect included cow. The LSMEANS option was used to calculate treatment means. The separation of main effects and any interactions was accomplished using the PDIFF statement.

RESULTS AND DISCUSSION

Serum Luteinizing Hormone

Mean serum LH concentration exhibited ($P = 0.02$; Table 1) an Arg \times tall fescue seed type interaction. Cows consuming toxic endophyte-infected tall fescue seed without Arg had decreased ($P < 0.05$) LH concentrations. However, when cows were fed toxic endophyte-infected tall fescue seed with Arg, serum LH concentrations were similar ($P \geq 0.67$) to those of cows consuming endophyte-free tall fescue seed with and without Arg. Postruminal infusion of Arg has been

Table 1. Effects of rumen-protected arginine and fescue seed type on serum LH dynamics in young beef cows

Measurement	RP-Arg ¹				SEM	P-value		
	0		180			Arg	Fescue	Arg × fescue
	Fescue seed ²							
	Endophyte free	Infected	Endophyte free	Infected				
LH mean, ng/mL	0.49 ^a	0.13 ^b	0.53 ^a	0.45 ^a	0.07	0.04	0.05	0.02
LH peaks, No.	8.3	5.8	7.8	7.9	0.80	0.48	0.09	0.16
LH amplitude, ng/mL	1.37 ^a	0.78 ^b	1.41 ^a	1.21 ^a	0.09	0.05	0.02	0.10

^{a,b}Means with different superscripts differ ($P \leq 0.05$).

¹Rumen-protected Arg (RP-Arg) supplement fed at 0 or 180 mg/kg of BW.

²Endophyte-free tall fescue seed supplement fed at 0.5 kg twice daily; toxic endophyte-infected tall fescue seed supplement fed at 0.5 kg twice daily.

reported to increase mean plasma LH concentration in prepubertal ewes (Recabarren et al., 1996). In the current study, mean LH was reduced ($P = 0.03$) in cows fed toxic endophyte-infected tall fescue seed compared to endophyte-free tall fescue seed; however, no difference ($P = 0.60$) was found between cows receiving Arg with either tall fescue seed type. Similarly, McKenzie and Erickson (1991) observed a 23% decrease in mean LH concentration in 3-mo-old heifers consuming toxic endophyte-infected tall fescue hay. Furthermore, LH concentrations were reduced after ergotamine treatment in steers (Browning et al., 1997), Holstein cows (Browning et al., 1998), and rats (Raj and Greep, 1973). Christopher et al. (1990) reported that gonadotropes were adversely affected when heifers consumed endophyte-infected tall fescue. There was no interaction ($P = 0.16$) of Arg and tall fescue seed type on the number of LH peaks; cows receiving toxic endophyte-infected tall fescue seed tended ($P = 0.09$) to have a lower number of LH peaks compared to those receiving endophyte-free tall fescue seed. In addition, the number of LH peaks was not influenced ($P = 0.48$) by inclusion of Arg in the diet. In contrast, ewes infused with Arg had a greater percentage of LH pulses that were >1 ng/mL than ewes not receiving Arg (Recabarren et al., 1996).

Luteinizing hormone amplitude tended ($P = 0.10$; Table 1) to be influenced by Arg × tall fescue seed type interaction. The tendency for the interaction was due to the effect that rumen-protected Arg had on cows fed toxic endophyte-infected tall fescue seed. Luteinizing hormone amplitude was reduced ($P < 0.01$) for cows receiving toxic endophyte-infected tall fescue seed compared to endophyte-free tall fescue seed cows or Arg-supplemented cows with and without toxic endophyte-infected seed. Similarly, Browning et al. (1997) reported a decrease in LH amplitude in steers after intravenous ergotamine tartrate infusions. Within the toxic endophyte-infected fescue seed treatments, rumen-protected Arg increased ($P = 0.04$) LH amplitude in cows fed toxic endophyte-infected tall fescue seed compared to cows receiving no rumen-protected Arg with toxic endophyte-infected tall fescue seed. In contrast, Recabarren

et al. (1996) reported no difference in mean LH pulse amplitude when prepubertal ewes received Arg infusions. These results indicate that Arg supplementation in young cows irrespective of toxic endophyte-infected tall fescue may stimulate LH secretion and therefore affect ovarian function and reproductive cyclicity.

Serum Nitric Oxide

Circulating NO concentrations tended to be influenced ($P = 0.09$; Table 2) by the interaction of Arg × tall fescue seed type. Nitric oxide concentrations were reduced ($P < 0.05$) for cows receiving toxic endophyte-infected tall fescue seed compared to cows receiving endophyte-free tall fescue seed or Arg-supplemented cows with and without toxic endophyte-infected seed. In cows receiving endophyte-free fescue seed, NO concentrations were not altered ($P = 0.68$) by the addition of rumen-protected Arg to the diet. In contrast, Morris et al. (2000) reported a significant increase in circulating NO concentrations in healthy adult humans receiving oral Arg supplementation. Furthermore, Arg supplementation in rats resulted in increased endothelial NO production (Kohli et al., 2004). However, Liu et al. (2009) reported no difference in NO production in adult male humans supplemented with Arg, but subjects received Arg for only 3 d prior to NO metabolite analysis. At normal physiological concentrations of Arg in healthy individuals, NO production may be limited because of saturated levels of NO synthase (Luiking et al., 2010). Thus, Arg supplementation may not increase NO synthase activity; consequently, no further NO production would be observed. In contrast, Al-Tamimi et al. (2007) reported a decrease in nitrate and nitrite concentrations in rats receiving toxic endophyte-infected tall fescue diets, indicating reduced endogenous NO production. However, the tendency for NO concentrations to be greater when cows received toxic endophyte-infected tall fescue seed with Arg may be explained by the fact that NO production is increased by Arg supplementation only in individuals with endothelial dysfunction (Alvares et al., 2012).

Table 2. Effects of rumen-protected arginine and fescue seed type on circulating serum nitric oxide in young beef cows

Measurement	RP-Arg ¹				SEM	P-value		
	0		180			Arg	Fescue	Arg × fescue
	Fescue seed ²							
	Endophyte free	Infected	Endophyte free	Infected				
Nitric oxide, μM	4.88 ^a	4.26 ^b	4.68 ^a	4.77 ^a	0.16	0.48	0.24	0.09

^{a,b}Means with different superscripts differ ($P \leq 0.05$).

¹Rumen-protected Arg (RP-Arg) supplement fed at 0 or 180 mg/kg of BW.

²Endophyte-free tall fescue seed supplement fed at 0.5 kg twice daily; toxic endophyte-infected tall fescue seed supplement fed at 0.5 kg twice daily.

Color Doppler Ultrasonography

Median caudal artery area ($P = 0.03$; Table 3) and diameter ($P = 0.01$) were reduced in cows receiving toxic endophyte-infected tall fescue seed compared to cows receiving endophyte-free tall fescue seed. Likewise, median caudal artery area has been reported to decrease for beef heifers receiving toxic endophyte-infected tall fescue seed compared to heifers receiving endophyte-free tall fescue seed (Aiken et al., 2007, 2009). Furthermore, Aiken et al. (2013) reported a decrease in median caudal artery area when steers grazed toxic endophyte-infected tall fescue compared to steers grazing endophyte-free tall fescue pastures. Osborn et al. (1992) reported a decrease in blood flow to various peripheral tissues in steers consuming a toxic endophyte-infected tall fescue diet during thermoneutral and elevated environment temperatures. This reduction in blood flow may lead to a reduced ability for the animal to dissipate heat, therefore leading to elevated peripheral and core body temperatures (Rhodes et al., 1991) and increased respiration rates. Rumen-protected Arg did not influence ($P \geq 0.84$) either mean caudal area or caudal diameter. In agreement, Yunta et al. (2015) reported no differences in uterine artery diameter in dairy heifers receiving daily intraperitoneal infusions of 40 mg Arg/kg BW.

Peak systolic velocity (PSV) did not differ ($P = 0.35$; Table 3) between tall fescue seed treatments. Likewise, Aiken et al. (2009) did not report a difference in PSV for beef heifers treated with toxic endophyte-infected tall fescue seed. In contrast, Aiken et al. (2007) reported an increase in PSV from baseline at 76 h in beef heifers after consumption of toxic endophyte-infected tall fescue seed compared to those consuming endophyte-free tall fescue seed. Furthermore, PSV did not differ ($P = 0.59$) when cows receiving rumen-protected Arg were compared to cows not receiving Arg. In contrast, PSV in the corpus luteum increased in ewes treated with 90 and 360 mg/kg BW of rumen-protected Arg compared to ewes not receiving rumen-protected Arg (Saevre et al., 2011). However, end diastolic velocity (EDV) was lower ($P = 0.05$) for cows receiving endophyte-free tall fescue seed than for those receiving toxic endophyte-

infected tall fescue seed. In contrast, Aiken et al. (2007) reported no difference in EDV in beef heifers receiving either toxic endophyte-infected or endophyte-free tall fescue seed. End diastolic velocity showed a tendency to decrease ($P = 0.09$) when cows received rumen-protected Arg. This tendency was driven by an increase in EDV for cows receiving toxic endophyte-infected fescue seed rather than Arg altering EDV. However, the Arg × fescue seed type interaction was not significant ($P = 0.14$) for EDV. Mean velocity was greater ($P = 0.01$) for cows receiving rumen-protected Arg compared to those that did not receive Arg. In agreement, Ohta et al. (2007) reported an increase in blood velocity in rats fed Arg at 50 and 150 mg/kg BW. Mean velocity did not differ ($P = 0.68$) for cows receiving toxic endophyte-infected tall fescue seed and those receiving endophyte-free tall fescue seed. Likewise, other studies reported that toxic endophyte-infected tall fescue seed did not influence mean velocity in beef heifers compared to those receiving endophyte-free tall fescue seed (Aiken et al., 2007, 2009).

Cows receiving toxic endophyte-infected tall fescue seed had a decreased ($P = 0.03$) resistance index (RI) compared to cows receiving endophyte-free tall fescue seed. Furthermore, RI was increased ($P = 0.05$) in cows receiving Arg compared to cows that did not receive Arg. In addition, pulsatility index (PI) was greater ($P = 0.03$) for heifers receiving endophyte-free tall fescue seed compared to those receiving toxic endophyte-infected tall fescue seed. However, Aiken et al. (2007) reported no difference in PI in heifers receiving toxic endophyte-infected tall fescue seed versus those receiving endophyte-free tall fescue seed. Furthermore, PI did not differ ($P = 0.15$) for cows receiving Arg compared to cows not receiving Arg. In contrast, rumen-protected Arg did not influence RI and PI in the CL or ovarian hilus in ewes (Saevre et al., 2011). Blood flow was influenced ($P = 0.05$) by the interaction of Arg × tall fescue seed type. Blood flow was unaffected by tall fescue seed type. Blood flow was increased in cows receiving Arg compared to cows that did not receive Arg. Overall, supplementing Arg improved the rate of blood flow in cows that

Table 3. Effects of rumen-protected arginine and fescue seed type on caudal blood flow parameters in young beef cows

Measurement	RP-Arg ¹				SEM	P-value		
	0		180			Arg	Fescue	Arg × fescue
	Fescue seed ²							
	Endophyte free	Infected	Endophyte free	Infected				
Area, mm ²	5.65	5.20	5.89	5.09	0.25	0.84	0.03	0.39
Diameter, mm	3.60	3.31	3.75	3.24	0.20	0.89	0.01	0.38
PSV, ³ cm/s	54.09	52.70	57.99	52.64	2.92	0.59	0.35	0.58
EDV, ⁴ cm/s	3.47	7.66	3.33	3.92	1.22	0.09	0.05	0.14
MNV, ⁵ cm/s	35.84	38.57	43.31	42.26	1.99	0.01	0.68	0.39
Resistance index ⁶	0.94	0.87	0.95	0.93	0.02	0.05	0.03	0.21
Pulsatility index ⁷	1.44	1.23	1.28	1.21	0.07	0.15	0.03	0.28
Blood flow, ⁸ mL/min	572 ^a	629 ^{a,b}	793 ^c	670 ^b	58	0.01	0.46	0.05

^{a-c}Means with different superscripts differ ($P \leq 0.05$).

¹Rumen-protected Arg (RP-Arg) supplement fed at 0 or 180 mg/kg of BW.

²Endophyte-free tall fescue seed supplement fed at 0.5 kg twice daily; toxic endophyte-infected tall fescue seed supplement fed at 0.5 kg twice daily.

³PSV = peak systolic velocity.

⁴EDV = end diastolic velocity.

⁵MNV = mean velocity.

⁶Resistance index (RI) was calculated as $RI = (PSV - EDV)/PSV$.

⁷Pulsatility index (PI) was calculated as $PI = (PSV - EDV)/MNV$.

⁸Blood flow was calculated as $BF = MNV \times \text{cross-sectional diameter} \times 60$.

received endophyte-free tall fescue seed compared to non-Arg endophyte-free tall fescue seed cows.

Caudal hemodynamics in the current study was altered more by fescue seed type than the additional rumen-protected Arg. As expected, cows receiving toxic endophyte-infected tall fescue seed experienced vasoconstriction. Furthermore, rumen-protected Arg did not alter caudal area or diameter. This response was unexpected because of the influence Arg has on NO synthesis. Overall, these results may indicate that either Arg supplementation was inadequate to elicit a vasodilation effect in cows consuming toxic endophyte-infected tall fescue seeds; however, Arg supplementation at 180 mg/kg BW did increase caudal blood flow.

Overall Conclusions

Supplementing young cows grazing toxic endophyte-infected tall fescue with rumen-protected arginine may offset negative effects of decreased blood flow and LH concentration. The inclusion of rumen-protected Arg with toxic endophyte-infected tall fescue seed led to an increase in artery blood velocity and serum LH concentrations. This increase in peripheral blood velocity may lead to increased nutrient and hormone uptake. The increase in LH during the follicular phase may provide an optimal environment for ovulation, oocyte quality, corpus luteum function, and subsequent embryonic development competence. Thus, this study indicates that supplying additional postprandial arginine in livestock grazing toxic endophyte-infected tall fescue may be ben-

eficial and potentially mitigate some negative effects of fescue toxicosis in regard to blood flow and cow fertility. However, future research will be needed to quantify if these results translate in an applied production setting.

LITERATURE CITED

- Aiken, G., B. Kirch, J. Strickland, L. Bush, M. Looper, and F. Schrick. 2007. Hemodynamic responses of the caudal artery to toxic tall fescue in beef heifers. *J. Anim. Sci.* 85:2337–2345. doi:10.2527/jas.2006-821
- Aiken, G., J. Klotz, J. Johnson, J. Strickland, and F. Schrick. 2013. Postgraze assessment of toxicosis symptoms for steers grazed on toxic endophyte-infected tall fescue pasture. *J. Anim. Sci.* 91:5878–5884. doi:10.2527/jas.2012-5964
- Aiken, G., J. Strickland, M. Looper, L. Bush, and F. Schrick. 2009. Hemodynamics are altered in the caudal artery of beef heifers fed different ergot alkaloid concentrations. *J. Anim. Sci.* 87:2142–2150. doi:10.2527/jas.2008-1562
- Al-Tamimi, H., P. Eichen, G. Rottinghaus, and D. Spiers. 2007. Nitric oxide supplementation alleviates hyperthermia induced by intake of ergopeptine alkaloids during chronic heat stress. *J. Therm. Biol.* 32:179–187. doi:10.1016/j.jtherbio.2006.11.003
- Alvares, T. S., C. A. Conte-Junior, J. T. Silva, and V. M. F. Paschoalin. 2012. Acute L-arginine supplementation does not increase nitric oxide production in healthy subjects. *Nutr. Metab.* 9:54. doi:10.1186/1743-7075-9-54
- Bossis, I., R. Wettemann, S. Welty, J. Vizcarra, L. Spicer, and M. Diskin. 1999. Nutritionally induced anovulation in beef heifers: Ovarian and endocrine function preceding cessation of ovulation. *J. Anim. Sci.* 77:1536–1546. doi:10.2527/1999.7761536x

- Browning, R., F. Schrick, F. Thompson, and T. Wakefield. 1998. Reproductive hormonal responses to ergotamine and ergonovine in cows during the luteal phase of the estrous cycle. *J. Anim. Sci.* 76:1448–1454. doi:10.2527/1998.7651448x
- Browning, R., F. Thompson, J. Sartin, and M. Leite-Browning. 1997. Plasma concentrations of prolactin, growth hormone, and luteinizing hormone in steers administered ergotamine or ergonovine. *J. Anim. Sci.* 75:796–802. doi:10.2527/1997.753796x
- Bussard, J. R. 2012. Evaluation of vascular changes in cattle relative to time-off endophyte-infected tall fescue. MS Thesis. Univ. of Kentucky, Lexington.
- Christopher, G., B. Salfen, S. Schmidt, J. Arbona, D. Marple, J. Sartin, D. Bransby, R. Carson, and C. Rahe. 1990. Effects of grazing Kentucky-31 tall fescue infected with *Acremonium coenophialium* on endocrine function in ovariectomized beef heifers. *J. Anim. Sci.* 68(Suppl. 1):469. (Abstr.)
- Coblentz, W. K., K. P. Coffey, T. F. Smith, D. S. Hubbell III, D. A. Scarbrough, J. B. Humphry, B. C. McGinley, J. E. Turner, J. A. Jennings, C. P. West, M. P. Popp, D. H. Hellwig, D. L. Kreider, and C. L. Rosenkrans Jr. 2006. Using orchardgrass and endophyte-free fescue versus endophyte-infected fescue overseeded on bermudagrass for cow herds: II. Four-year summary of cow-calf performance. *Crop Sci.* 46:1929–1938. doi:10.2135/cropsci2005.11-0443
- Donoso, A. O., F. J. López, and A. Negro-Vilar. 1990. Glutamate receptors of the non-N-methyl-D-aspartic acid type mediate the increase in luteinizing hormone-releasing hormone release by excitatory amino acids in vitro. *Endocrinology* 126:414–420. doi:10.1210/endo-126-1-414
- Dyer, D. C. 1993. Evidence that ergovaline acts on serotonin receptors. *Life Sci.* 53:PL223–PL228. doi:10.1016/0024-3205(93)90555-H
- Gay, N., J. Boling, R. Dew, and D. Miksch. 1988. Effects of endophyte-infected tall fescue on beef cow-calf performance. *Appl. Agric. Res.* 3:182–186.
- Goodman, R. L., and F. J. Karsch. 1980. Pulsatile secretion of luteinizing hormone: Differential suppression by ovarian steroids. *Endocrinology* 107:1286–1290. doi:10.1210/endo-107-5-1286
- Hoveland, C. S. 1993. Importance and economic significance of the *Acremonium endophytes* to performance of animals and grass plant. *Agric. Ecosyst. Environ.* 44:3–12. doi:10.1016/0167-8809(93)90036-O
- Kohli, R., C. J. Meininger, T. E. Haynes, W. Yan, J. T. Self, and G. Wu. 2004. Dietary L-arginine supplementation enhances endothelial nitric oxide synthesis in streptozotocin-induced diabetic rats. *J. Nutr.* 134:600–608.
- Liu, T. H., C.-L. Wu, C.-W. Chiang, Y.-W. Lo, H.-F. Tseng, and C.-K. Chang. 2009. No effect of short-term arginine supplementation on nitric oxide production, metabolism and performance in intermittent exercise in athletes. *J. Nutr. Biochem.* 20:462–468. doi:10.1016/j.jnutbio.2008.05.005
- Luiking, Y. C., M. P. K. J. Engelen, and N. E. P. Deutz. 2010. Regulation of nitric oxide production in health and disease. *Curr. Opin. Clin. Nutr. Metab. Care* 13:97–104. doi:10.1097/MCO.0b013e328332f99d
- McKenzie, P., and B. Erickson. 1991. Effects of fungal-infested fescue on gonadotrophin secretion and folliculogenesis in beef heifers. *J. Anim. Sci.* 69(Suppl. 1):387. (Abstr.)
- Meyer, A. M., C. B. Saevre, D. V. Dhuyvetter, R. E. Musser, and J. S. Caton. 2011. Effects of rumen protected arginine supplementation on serum amino acid concentrations in forage-fed steer. *Proc. West Sect. Am. Soc. Anim. Sci.* 62:368–371.
- Miller, N. P. 2012. Utilization of dried distillers grains to manage rate of body weight gain during heifer development and the use of a novel rumen-protected arginine to alter plasma levels of arginine. MS Thesis. New Mexico State Univ., Las Cruces.
- Morris, C. R., F. A. Kuypers, S. Larkin, N. Sweeters, J. Simon, E. P. Vichinsky, and L. A. Styles. 2000. Arginine therapy: A novel strategy to induce nitric oxide production in sickle cell disease. *Br. J. Haematol.* 111:498–500. doi:10.1111/j.1365-2141.2000.02403.x
- Moura, A. A., and B. H. Erickson. 1997. Age-related changes in peripheral hormone concentrations and their relationships with testis size and number of Sertoli and germ cells in yearling beef bulls. *J. Reprod. Fertil.* 111:183–190.
- Ohta, F., T. Takagi, H. Sato, and L. J. Ignarro. 2007. Low dose L-arginine administration increases microperfusion of hindlimb muscle without affecting blood pressure in rats. *Proc. Natl. Acad. Sci. U.S.A.* 104:1407–1411. doi:10.1073/pnas.0610207104
- Osborn, T., S. Schmidt, D. Marple, C. Rahe, and J. Steenstra. 1992. Effect of consuming fungus-infected and fungus-free tall fescue and ergotamine tartrate on selected physiological variables of cattle in environmentally controlled conditions. *J. Anim. Sci.* 70:2501–2509. doi:10.2527/1992.7082501x
- Peters, C. W., K. N. Grigsby, C. G. Aldrich, J. A. Paterson, R. J. Lipsey, M. S. Kerley, and G. B. Garner. 1992. Performance, forage utilization, and ergovaline consumption by beef-cows grazing endophyte fungus-infected tall fescue, endophyte fungus-free tall fescue, or orchardgrass pastures. *J. Anim. Sci.* 70:1550–1561. doi:10.2527/1992.7051550x
- Petersen, L., J. Petersen, U. Talleruphuus, S. Ladefoged, J. Mehlsen, and H. Jensen. 1997. The pulsatility index and the resistive index in renal arteries. Associations with long-term progression in chronic renal failure. *Nephrol. Dial. Transplant.* 12:1376–1380. doi:10.1093/ndt/12.7.1376
- Raj, H. G. M., and R. O. Greep. 1973. Inhibition of ovulation and luteinizing-hormone secretion in cyclic rat by ergotamine tartrate. *Proc. Soc. Exp. Biol. Med.* 144:960–962. doi:10.3181/00379727-144-37720
- Recabarren, S. E., A. Jofré, A. Lobos, P. Orellana, and J. Parilo. 1996. Effect of arginine and ornithine infusions on luteinizing hormone secretion in prepubertal ewes. *J. Anim. Sci.* 74:162–166. doi:10.2527/1996.741162x
- Rhodes, F., L. Fitzpatrick, K. Entwistle, and G. De'Ath. 1995. Sequential changes in ovarian follicular dynamics in *Bos indicus* heifers before and after nutritional anoestrus. *J. Reprod. Fertil.* 104:41–49. doi:10.1530/jrf.0.1040041
- Rhodes, M., J. Paterson, M. Kerley, H. Garner, and M. Laughlin. 1991. Reduced blood flow to peripheral and core body tissues in sheep and cattle induced by endophyte-infected tall fescue. *J. Anim. Sci.* 69:2033–2043. doi:10.2527/1991.6952033x
- Saevre, C. B., J. S. Caton, J. S. Luther, A. M. Meyer, D. V. Dhuyvetter, R. E. Musser, J. D. Kirsch, M. Kapphahn, D. A. Redmer, and C. S. Schauer. 2011. Effects of rumen-protected arginine supplementation on ewe serum-amino-acid concentration, circulating progesterone, and ovarian blood flow. *Sheep Goat Res. J.* 46:8–12.
- Schuenemann, G. M., M. E. Hockett, J. L. Edwards, N. R. Rohrbach, K. F. Breuel, and F. N. Schrick. 2005. Embryo development and survival in beef cattle administered ergotamine tartrate to simulate fescue toxicosis. *Reprod. Biol.* 5:137–150.

- Sleper, D., and C. West. 1996. Tall fescue. In: L. E. Moser, D. R. Buxton, and M. D. Caster, editors, *Cool-season forage grasses*. Agron. Monogr. No. 34. Am. Soc. Agron., Crop Sci. Soc. Am., Soil Sci. Soc. Am., Madison, WI. p. 471–505.
- Thompson, F., J. Stuedemann, and N. Hill. 2001. Anti-quality factors associated with alkaloids in eastern temperate pasture. *J. Range Manage.* 54:474–489. doi:10.2307/4003119
- Wu, G., and J. S. Morris. 1998. Arginine metabolism: Nitric oxide and beyond. *Biochem. J.* 336:1–17. doi:10.1042/bj3360001
- Yates, S. G., and R. G. Powell. 1988. Analysis of ergopeptine alkaloids in endophyte-infected tall fescue. *J. Agric. Food Chem.* 36:337–340. doi:10.1021/jf00080a023
- Yunta, C., K. A. Vonnahme, B. R. Mordhost, D. M. Hallford, C. O. Lemley, C. Parys, and A. Bach. 2015. Arginine supplementation between 41 and 146 days of pregnancy reduces uterine blood flow in dairy heifers. *Theriogenology* 84:43–50. doi:10.1016/j.theriogenology.2015.02.007