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Ruminant Nutrition

RUMINANT NUTRITION

Ruminal motility, reticuloruminal fill, and eating patterns in steers exposed to ergovaline

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Abstract

Fescue toxicosis is problematic for growing steers, causing lower DMI and productivity when fed endophyte-infected (E+) tall fescue. A complete understanding of underlying mechanisms of how fescue toxicosis affects growing steers is lacking. Therefore, the overall objective of this multiexperiment study was to determine whether ruminally dosed ergovaline (**ERV**) affects rumen motility, rumen contents, and eating patterns. In Exp. 1, an 8-h period to assess ruminal motility began 4 h after feeding by monitoring pressure changes using a wireless system for 21 d. Eight ruminally cannulated steers (283 kg BW) were pair fed with alfalfa cubes (1.5 × NE_m) and assigned to endophyte free (E–; 0 μg ERV/kg BW/d) or E+ treatment (20 μg ERV/kg BW/d). Overall, E+ steers had more frequent rumen contractions (Seed $P = 0.05$ and day of feeding $P = 0.02$). On days 7 to 9, both treatments showed lower frequencies and E– steers had greater amplitude of contractions ($P < 0.001$) that corresponded with decreased DMI. In Exp. 2, steers remained in pairs assigned in Exp. 1 (322 kg BW), but reversed seed treatments while increasing ERV levels (titrated 0, 5, 10, 15, and 20 μg ERV/kg BW/d over 57 d). There were no differences between E– and E+ for frequency ($P = 0.137$) or amplitude of contractions ($P = 0.951$), but increasing ERV dosage, decreased frequency ($P = 0.018$) and amplitude ($P = 0.005$), coinciding with lower DMI. In Exp. 3, 8 steers (589 kg) were pair fed and ruminally dosed 15 μg ERV/kg BW/d, and rumen motility data were collected for 21 d. E– steers showed higher amplitude and lower frequency of contractions than E+ steers with seed ($P < 0.001$), day ($P < 0.001$), and seed × day ($P < 0.04$) effects, but rumen fill was not different between E– and E+ ($P > 0.29$). Serum prolactin concentrations were lower in E+ steers in Exp. 1 to 3. Eating patterns of pair-fed E– and E+ steers were relatively slower in E+ than E– (Exp. 4) by measuring every 2 h across 24 h. Number of meals were higher in E+ than E– steers, but meal duration and meal size were not different between treatments. Rumen content (DM%) tended to be higher in E+ than in E– when steers were fed once a day ($P = 0.07$), but there was no difference for rumen content (DM%) when E– and E+ steers were fed 12 times a day ($P = 0.13$). These results suggest the changes in rumen fill associated with fescue toxicosis may be driven more by changes in feeding behavior and eating pattern rather than by changes in motility.

Key words: bovine, eating patterns, ergot alkaloids, fescue toxicosis, rumen motility, ruminant

Introduction

Fescue toxicosis is one of the largest health-related production costs for the grazing livestock industry, with costs exceeding 2 billion dollars annually (Kallenbach, 2015). Losses are due to hyperthermia, rough hair coat, reduced intake and gain, lowered calving rates, agalactia, and hypoprolactinemia (Schmidt and Osborn, 1993).

When steers were fed endophyte-infected tall fescue seed, the DM and total DM weight of ruminal contents were increased in endophyte-infected (E+) treatments (Foote et al., 2013; Koontz et al., 2013). Due to pair feeding by Koontz et al. (2013) and Foote et al. (2013), there were no intake differences between endophyte-free E– and E+ treatments, indicating that the observed increase in ruminal fill by dosing E+ seed may originate from other factors such as alteration of rumen kinetics or eating patterns. Egert et al. (2014) determined real-time rumen motility patterns relative to feeding in steers. Using steers receiving 10 μg ergovaline (**ERV**)/kg BW per day at thermoneutral conditions for 14 d, Egert et al. (2014) observed no differences in ruminal motility, fill (kg), or DM of contents. However, the severity of fescue toxicosis was unclear. Consequently, it was hypothesized that ergot alkaloid dose and dose interval may alter the impact of ergot alkaloids on rumen motility. Furthermore, it is not known how eating patterns of pair-fed steers treated with E– vs. E+ seed are affected.

To examine these concepts, a series of 4 experiments were conducted. The objectives of these experiments were to examine reticuloruminal motility patterns and reticulorumen contents from steers subjected to a high ERV dosage for 21 d (20 μg ERV/kg BW/d; Exp. 1) and gradually increasing dosages of ERV for 57 d (0, 5, 10, 15, and 20 μg ERV/kg BW/d; Exp. 2); to examine reticuloruminal motility patterns and reticulorumen contents with an intermediate dosage (15 μg ERV/kg BW/d; Exp 3) for 21 d; and to determine eating patterns of steers fed E– and E+ seeds (10 μg ERV/kg BW/d; Exp. 4).

Materials and Methods

The procedures used in this study were approved by the University of Kentucky Institutional Animal Care and Use Committee. All experiments were conducted at the University of Kentucky C. Oran Little Research Center in Versailles, KY, under thermoneutral conditions.

Experiment 1. Rumen Motility Measured by Wireless Telemetry System and Reticuloruminal Fill in Steers Exposed to High Ergovaline

Animal management and experimental design

Eight ruminally cannulated Holstein steers (BW = 283 ± 11.8 kg) were fed a diet of alfalfa cubes (% composition on a DM basis: CP = 17.1; ADF = 39.5; NDF = 47.8; NFC = 21.1; TDN = 57; NE_m = 4.89 MJ/kg) at 1.5 × NE_m once daily (0800 h). Feed was top-dressed with a trace mineral premix (Kentucky Nutrition Service, Lawrenceburg, KY; composition: NaCl = 92% to 96%; Fe = 9,275 ppm; Zn = 5,500 ppm; Mn = 4,790 ppm; Cu = 1,835 ppm; I = 115 ppm; Se = 18 ppm; Co = 65 ppm) to meet nutrient requirements by NRC (2000). Steers were housed indoors at 22 °C in 3 × 3 m individual stalls with ad libitum access to water.

Animals were blocked by weight in a randomized complete block design and randomly assigned to either E– (KY 32; 0 mg ERV/kg DM) or E+ (KY 31; lot 1 = 4.64 mg ERV/kg DM; lot 2 = 2.33 mg ERV/kg DM) seed treatment within a block. The E– steers received 0 μg ERV/kg BW/d and E+ steers received 20 μg ERV/kg BW/d. A combination of E– and E+ seed was used to ensure equivalent seed weight (1.29 kg daily) to all steers. Pair feeding was used to maintain intake consistent within a block; thus, the E– steers received the same amount of feed the paired E+ steers consumed the previous day.

Reticuloruminal motility measurements

A wireless telemetry system was used (emkaPACK4G telemetry system; emka Technologies, Falls Church, VA) for monitoring real-time pressure changes within the rumen as described by Egert et al. (2014). The system consists of 3 parts: 1) a balloon connected to a transducer, 2) transducer and transmitter for detecting pressure and sending data to receiver, and 3) wireless receiver. A water-filled (warm tap water, 2 L) latex balloon (60.9 cm; Party Magic USA, Bucks County, PA) connected to a pressure transducer with Tygon tubing (25 cm, i.d. = 3.2 mm; o.d. = 6.4 mm) was placed in the ventral sac of the rumen to measure reticuloruminal motility. During collection periods, the transducer and transmitter were housed in a square-shaped plastic airtight container (1.28 L; World Kitchen, LLC, Rosemont, IL) and secured near the withers with wires. The wireless receivers mounted securely at the wall of outside of pen and were hardwired to a 8-channel PO E+ switch (8-port gigabit GREENnet PO E+ switch, TRENDnet, Torrance, CA), which was connected to a laptop. The recorded data were analyzed for amplitude (mm Hg) and frequency (contractions/min).

Sample and data collection

Reticuloruminal motility data were collected over the 21 d of ruminal seed dosing, an 8-h data collection period began 4 h after feeding every other day (days 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, and 21). Data were recorded and stored using iox2 (iox 2.9.4.27, emka Technologies) software with a sampling rate of 100 pressure readings per second. On days 1, 7, 14 and 22, blood (approximately 10 mL) was collected via jugular veinipuncture immediately using nonheparinized vacuum tubes before dosing seed. Blood samples were allowed to clot for 24 h at 4 °C and then centrifuged at 1,500 × *g* for 25 min (4 °C). Collected serum was stored at –20 °C. Serum prolactin concentrations were analyzed via radioimmunoassay as described in Bernard et al. (1993). Interassay and intra-assay were 4.42% and 7.35%, respectively.

Rumen evacuation was performed on day 22. Prior to feeding time (0800 h), reticulorumen contents were emptied manually through the cannula, mixed, and weighed. Ruminal fill was determined for each steer via weight of ruminal contents. Triple samples (approximately 100 g from each animal) were dried in a 55 °C oven overnight for DM analysis. The remaining contents were returned to the rumen immediately after sampling. Water intake was recorded using analog water flow meter (DLJ Multi-Jet Water Meter, Daniel L. Jerman Co., Hackensack, NJ) immediately before feeding (0800 h), prior to motility data collection (1200 h), and at the end of collection (2000 h).

Experiment 2. Rumen Motility and Reticuloruminal Fill in Steers Exposed to Increasing Ergovaline

Animal management and experimental design

The same 8 ruminally cannulated Holstein steers (BW = 322 kg ± 18.5 kg) from Exp. 1 remained in pairs for this experiment. After a 42-d washout period, seed treatments were reversed and control steers from Exp. 1 received E+ seed in Exp. 2. The same E– and E+ seed lots from Exp. 1 were used in Exp. 2. The E+ steers received 5 μg ERV/kg BW/d for days 1 to 14; 10 μg ERV/kg BW/d for days 15 to 28; 15 μg ERV/kg BW/d for days 29 to 43; and received 20 μg ERV/kg BW/d for days 44 to 57. E– steers received 0 μg ERV/kg BW/d for days 1 to 57. Cattle were weighed on days 15, 29, and 44 to adjust seed weight to meet dose requirement on a BW basis.

All steers were ruminally dosed with 1.46 kg (days 1 to 43) or 1.67 kg (days 44 to 57) of tall fescue seed. Total seed weight

increased due to increasing weight of cattle and a need to maintain seed weights constant across steers. A combination of E– and E+ seed was used to achieve proper dosage and ensure equivalent seed weights across individual steers. Steers were pair fed the same alfalfa cube diet as the previous experiment (Exp. 1) at 1.5 × NE_m once daily (0800 h), and the management of steers for this experiment was as described in Exp. 1.

Sample and data collection

To measure reticuloruminal motility, the wireless telemetry system (emka) and signal calibration used in this experiment were the same as described previously for Exp. 1. Over the 57 d of ruminal seed dosing, an 8-h data collection period began 4 h after feeding at the end of each dosing period (days 13, 14, 27, 28, 41, 42, 43, 56, and 57). Blood was collected on days 1, 15, 29, 44, and 58, blood (approximately 10 mL) for prolactin analysis as described in Exp. 1, and ruminal evacuations were performed on days 15, 29, 44, and 58 before feeding (0800 h). Rumen contents were emptied manually through the cannula, mixed, and weighed. Ruminal fill and DM analysis procedures were as described for Exp. 1.

Experiment 3. Rumen Motility Measured by Wired Telemetry System and Reticuloruminal Fill in Steers Exposed to Intermediate Dosage of Ergovaline

Animal management and experiment design

Eight Holstein steers (BW = 589 ± 18.6 kg), fitted with rumen cannulas were blocked by weight in a randomized complete block design. The experimental design was as described in Exp 1 and 2. Steers receiving E+ seed received 15 μg ERV/kg BW/d for 21 d. A combination of E– and E+ seed was used to ensure equivalent seed weight (2.41 kg) to all steers.

Reticuloruminal motility measurements and data analysis

A wired telemetry system connected to a data logger (Powerlab 8/30, ADInstruments Inc., Colorado Springs, CO) was used to characterize reticular contractions using the method developed and described by Egert et al. (2014) with modifications. A water-filled (500 mL) balloon attached to a Tygon catheter (i.d. = 3.2 mm; o.d. = 6.4 mm) was inserted into the ventral sac prior to feeding. Balloons were weighed to maintain consistent fill between animals and replaced if equipment failure occurred. A small hole was made in the plug of the rumen cannula to allow the catheter to pass through. The end of the catheter external to the animal was connected to a disposable blood pressure transducer (MLT0670, ADInstruments). All data were acquired using an analog-to-digital converter interfaced with a computer and was subsequently analyzed using commercially available software (LabChart 8, ADInstruments). The system was calibrated daily using a standard blood pressure manometer and rumen motility data were analyzed for contraction base, peak (mm Hg), amplitude (mm Hg), frequency (contractions/min), and durations (s).

Sample and data collection

Over the 21 d of ruminal seed dosing, an 8-h data collection period began 2 h after feeding every other day (on days 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, and 21) for collecting reticular motility data. Water intake was recorded using water flow meters immediately before feeding (0800 h), prior to collection (1000 h), and at the end of collection (1800 h). Blood samples were collected (approximately 10 mL) on days 0, 7, 15, and 22, from the jugular vein as described in Exp. 1 for serum prolactin analysis. Interassay and intra-assay were 4.24% and 5.38%, respectively.

Experiment 4. Eating Patterns and Reticuloruminal Fill in Steers Exposed to Ergovaline

Animal management experiment design

Eight ruminally cannulated steers (BW 560 ± 42.0 kg) were paired by weight in a randomized complete block design, for a 20-d experiment. During the last 4 d of a 14-d adaptation period, eating patterns were recorded for pair-fed E– and E+ steers. This was followed by a 6-d feeding simulation experiment where E– steers were pair fed 12 times/d to match the eating patterns of their E+ cohorts. Treatments for the adaptation period and feeding simulation were as follows: E– (KY-32; 0 mg ERV/kg DM) or E+ (KY-31; 4.64 mg ERV/kg DM) seed. Each E+ steer was ruminally dosed with 10 μg ERV/kg BW/d of E+ seed, and a paired E– steer was ruminally dosed with the same mass of E– seed (1.206 kg/d of each seed). All seed was ground to pass through a 3-mm screen. Animals were pair fed as described in Exp. 1 once daily at 0800 h to the quantity that the E+ steer consumed the previous day. Feed was top-dressed with a trace mineral premix (same as described in Exp. 1), and steers were housed as described above with ad libitum access to water.

Sample and data collection

Individual feed bunks were used to measure feed consumption of each steer. Each feed bunk was wired to a weighing sensor (load cell; RB-Phi-204, Phidgets Inc., Calgary, Canada), and all load cells were connected to a data logger (CR1000; Campbell Scientific, Logan, UT). Feed consumption was measured, recorded, and stored at every minute and collected every day for pair feeding prior to feeding at 0800 h. The number of meals per day, meal duration (min), average meal size (kg), and summary of meal sizes (kg) were calculated by individual feed consumption data using Matlab software (The MathWorks Inc., Natick, MA). Feed consumption data were imported into Matlab, smoothed using a median filter (order = 20), and analyzed using an algorithm where meals were characterized by a period of continuous feeding behavior or weight changes (Egert-McLean et al., 2019).

There were 10 d of adaptation prior to feed and water consumption (using analog water flow meter; DLJ Multi-Jet Water Meter, Daniel L. Jerman Co., Hackensack, NJ) being recorded for the last 96 h of the 14-d adaptation. This intake information was used to construct a feeding simulation, which occurred immediately after the adaptation period by using automatic feeders (Ankom Technology, Macedon, NY). The feeders contained 12 cylinders with drop-down doors permitting the automated feeding of 12 separate meals. The E– and E+ steers received feed 12 times a day every 2 h, to simulate the eating patterns of E+ steers. Rumen evacuation (as the same method as Exp. 1) was performed at the end of adaptation period (day 15, before initiating the feeding simulation) and a day after feeding simulation (day 21).

Analysis of Feeds and Tall Fescue Seed

In this series of studies (Exp. 1 to 4), all feed samples were analyzed for CP (AOAC, 2012), ADF (ANKOM, 2011a), and NDF (ANKOM, 2011b) by the Forage Testing Laboratory (Dairy One, Inc., Ithaca, NY). Tall fescue seed was analyzed for ergovaline, ergovalinine, ergotamine, and ergotaminine content using high-performance liquid chromatography and fluorescence detection

Table 1. Mean results for the high-ergovaline dosage trial (Exp. 1) on DMI, water consumption, serum prolactin concentration, rumen contents, and reticuloruminal motility contraction variables measured for 21 d in E– and E+ tall fescue seed-treated steers

	Treatment			P-value		
Item	E–[1]	E+[2]	SEM[3]	Seed	Day	Seed × day
DMI, kg	5.76	5.72	0.24	0.902	<0.001	0.013
24-h water intake, L	26.71	21.92	3.17	0.363	<0.001	0.641
Water intake (0800 to 1200 h), L	14.55	5.13	1.53	0.022	<0.001	0.002
Water intake (1200 to 2000 h), L	11.08	10.41	1.35	0.751	<0.001	0.395
Serum prolactin, ng/mL	22.69	6.74	6.24	0.121	0.052	0.012
Rumen content, kg	35.15	32.64	0.99	0.171	—	—
Rumen content, DM %	11.73	17.56	1.25	0.046	—	—
Rumen DM content, kg	4.11	5.81	0.37	0.049	—	—
Rumen wet content, kg	31.04	26.83	1.33	0.112	—	—
g rumen content DM per kg BW	14.14	19.98	1.44	0.064	—	—
g rumen content DM per kg DMI	643.3	902.4	60.59	0.057	—	—
Amplitude, mm Hg	8.15	8.11	0.28	0.928	0.334	0.023
Frequency, contractions/min	0.96	1.27	0.07	0.051	0.020	0.683

[1]E–, endophyte-free tall fescue seed.
[2]E+, endophyte-infected tall fescue seed; 20 µg ERV/kg BW/d.
[3]SEM, Standard error of the mean, $n = 4$ steers per treatment.

procedure described in Carter et al. (2010) with modifications described in Klotz et al. (2018).

Statistical Analysis

SAS 9.4 (SAS Inst. Inc. Cary, NC) was used to analyze the data. For Exp. 1 to 3, values for amplitude and frequency were collected for each animal hourly and were averaged for each day. Averages were analyzed as a randomized complete block design for the effects of seed, day, and the seed × day interaction. Mean values for the variables (serum prolactin, frequency, amplitude, DMI, and water consumption) were analyzed using Proc GLIMMIX considering animal a random effect with day the repeated measure, with lowest Bayesian information criterion used to determine the best model for covariance structure. Measurements of ruminal content were analyzed as a randomized complete block design for seed effect. For Exp. 2, orthogonal polynomial contrasts in SAS 9.4 with animals as a random effect and dose and seed treatment as fixed effects were used to determine effect of dose on frequency of contractions, amplitude of contractions, and ruminal content measurements. For Exp. 4, SAS 9.4 was also used to calculate eating patterns, meal size, and rumen fill. Feed and water consumption were analyzed as a randomized complete block with repeated measures for the effects of seed. Results were considered significant at $P \leq 0.05$ and considered a trend at $0.05 < P \leq 0.10$.

Results

Experiment 1. Rumen Motility Measured by Wireless Telemetry System and Reticuloruminal Fill in Steers Exposed to High Ergovaline

There was no difference ($P > 0.05$) in DMI between treatments due to pair feeding (Table 1). However, DMI differed by day ($P < 0.001$), with day 7 having the lowest intake (4.04 ± 0.65 kg; Fig. 1) and seed × day effect ($P = 0.013$) with E+ steers consuming more than E– steers on day 11 despite the pair feeding.

Daily water intake was not different between seed and seed × day. Average 24-h water intake differed over the feeding period (day effect, $P < 0.001$) with day 7 having the lowest consumption (18.51 ± 2.44 L consumed), related to the lower DMI on day 7 (data not shown). Water intake after the morning feeding and before beginning ruminal motility collection (0800 to 1200 h) was higher in E– than E+ steers (seed, $P = 0.022$). There was a seed × day effect ($P = 0.002$) on water consumption before motility collection with E– steers increasing intake as trial days progressed. Water intake during the motility collection (1200 to 2000 h) was lowest on day 7 (day, $P < 0.001$), but was not different between treatments.

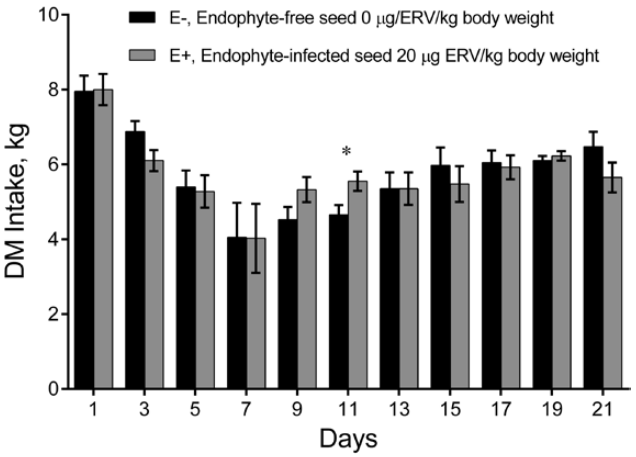

Figure 1. Daily DMI measured in the high-ergovaline dosage trial (Exp. 1) for 21 d in E– and E+ tall fescue seed-treated steers. Means ± SEM, $n = 4$. *Statistical difference ($P < 0.05$) between treatments within day. Seed × Day: $P = 0.013$.

Serum prolactin concentrations were lower for E+ steers on days 7, 14, and 21 (values were 24.7 and 27.1, 1.2 and 7.1, 0.6 and 7.1, 0.5 and 18.9 for E+ and E– on days 1, 7, 14, and 21, respectively) as the concentration decreased with days on experiment (seed × day effect $P = 0.012$).

The DM percentage of rumen contents of E+ steers (DM basis) was higher ($P = 0.046$) than E– steers (DM basis). Likewise, E+ steers had greater rumen DM contents ($P = 0.049$) compared with E– steers. There was a tendency ($P = 0.064$ and $P = 0.057$, respectively) for both g rumen content DM per kg BW and g

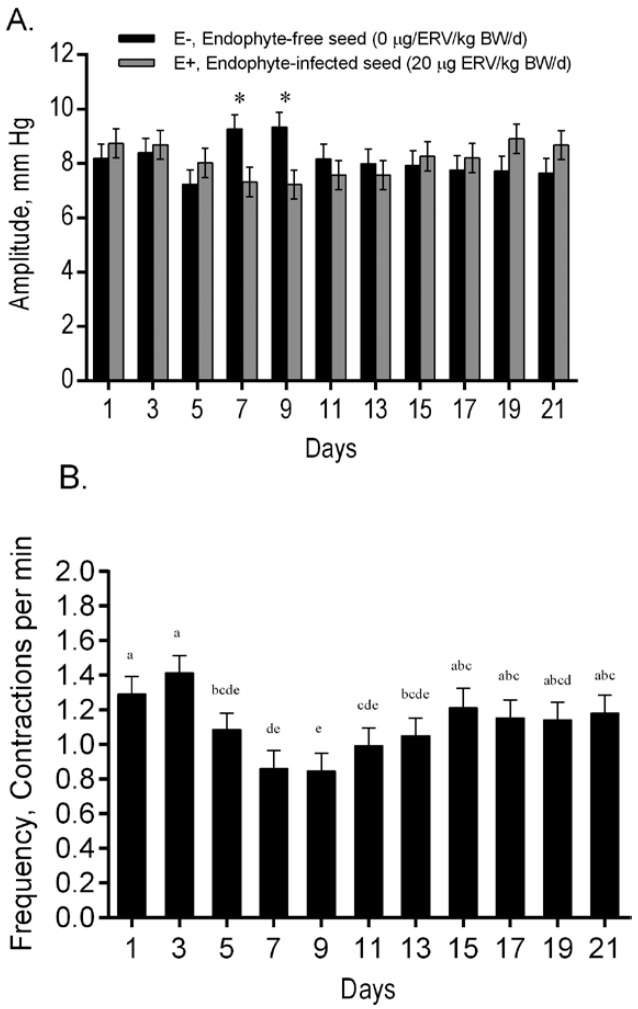

Figure 2. Ruminal contraction variables of steers for the high-ergovaline dosage trial (Exp. 1) for 21 d and ruminally dosed with E– and E+ ground tall fescue seed. Means ± SEM, $n = 4$. For (A), days denoted with "*" indicate difference ($P < 0.05$) between treatments, Seed × Day: $P = 0.023$. For (B), bars without a common superscript denote difference ($P < 0.05$) between days, Day: $P = 0.020$.

rumen content DM per kg intake to be higher for steers on E+ treatment.

There were no differences for seed and day on amplitude of ruminal contraction in E– and E+ steers. However, there was a seed × day interaction ($P = 0.023$) on amplitude with days 7 and 9, being higher in E– than E+ steers (Fig. 2A). A day effect was seen on frequency of contractions ($P = 0.020$) with both treatments having the lowest frequency of contractions on day 7 (0.84 contractions/min, Fig. 2B). There were seed ($P = 0.05$) and day ($P = 0.02$) effects on frequency of contractions, with E+ steers having a higher frequency than E– steers.

Experiment 2. Rumen Motility and Reticuloruminal Fill in Steers Exposed to Increasing Ergovaline

There was no difference in DMI ($P = 0.962$) between seed type (E– or E+ seed) due to pair feeding (Table 2). However, there was a dose effect ($P = 0.046$) with a decrease in DMI seen at the higher dosages of ERV (Fig. 3). Daily water intake was not different between seed types ($P = 0.751$); however, daily water intake increased linearly with increased dose of ERV ($P < 0.001$; 24-h water intake = 36.05 + 0.4205 × dose, $R^2 = 0.843$; data not shown). Water intake from the morning feeding before motility data collection (0800 to 1200 h) was higher in E– steers than in E+ steers, but there were no differences across seed type ($P = 0.490$), seed dose ($P = 0.808$), and seed × dose interaction ($P = 0.716$).

Water intake during motility data collection (1200 to 2000 h) was different between E– and E+ steers with dose ($P = 0.048$) and seed × dose interactions ($P = 0.001$) with E+ steers linearly [E+ water intake (1200 to 2000 h) = 16.10 + 0.33 × dose, $R^2 = 0.716$; data not shown] increasing water consumption compared with E– as dose level increased. Serum prolactin tended ($P = 0.106$) to be higher in E– steers than in E+ steers.

The total weight of rumen contents was higher in E+ steers than in E– steers, showing a tendency for a seed × dose interaction ($P = 0.062$; Table 2) with a linear trend ($P = 0.011$; Table 3) seen in the E+ steers where total weight of ruminal fill increased as dose increased. Rumen DM content weight showed a seed × dose interaction ($P = 0.05$), with a linear tendency for a decreasing trend ($P = 0.062$) as dose level increased in the E– steers (Fig. 4A). Higher rumen DM content weights were observed in E+ compared with E– steers dosed with 15 and 20 μg ERV/kg

Table 2. Mean results for the ergovaline dosage trial (Exp. 2) on DMI, water consumption, serum prolactin concentration, and reticuloruminal motility contraction variables measured for 57 d in E– and E+ tall fescue seed-treated steers

	Treatment			P-value		
Item	E–[1]	E+[2]	SEM[3]	Seed	Dose	Seed × dose
DMI, kg	8.15	8.14	0.19	0.962	0.046	0.351
24-h water intake, L	39.94	42.68	4.68	0.751	<0.001	0.302
Water intake (0800 to 1200 h), L	21.50	13.29	5.63	0.490	0.808	0.716
Water intake (1200 to 2000 h), L	15.96	20.23	4.09	0.595	0.048	0.001
Serum prolactin, ng/mL	39.95	3.54	7.27	0.106	0.315	0.281
Rumen content, kg	37.75	42.46	1.41	0.254	0.551	0.062
Rumen content, DM%	14.41	15.14	1.31	0.756	0.192	0.979
Rumen DM content, kg	5.47	6.37	0.27	0.257	0.197	0.052
Rumen wet content, kg	32.28	36.10	1.68	0.354	0.524	0.109
g rumen content DM per kg BW	15.21	17.28	1.28	0.456	0.022	0.011
g rumen content DM per kg DMI	671.4	792.7	27.16	0.195	0.045	0.011

[1]E–, endophyte-free tall fescue seed.
[2]E+, Endophyte-infected tall fescue seed; 5, 10, 15 and 20 μg ERV/kg BW/d
[3]SEM, standard error of the mean, $n = 4$ steers per treatment.

BW/d. There was a seed × dose interaction ($P = 0.011$) on g rumen DM per kg BW (Fig. 4B), with a tendency ($P = 0.076$) for a linear decrease in E– steers as dose level increased. A seed × dose level interaction ($P = 0.011$) was also seen on g rumen DM per kg DMI with a quadratic increase (Fig. 4C) in the E+ steers with increasing g rumen DM per kg DMI with ERV dose increased (E+ g rumen content DM per kg DMI = 939.22 – 250.77 × dose + 63.46 × dose^2; $R^2 = 0.0999$).

There was no seed and seed × dose effect of treatment ($P = 0.951$ and 0.309, respectively) on amplitude of contractions (Table 4, Fig. 5A); however, a dose effect was observed ($P = 0.005$). Amplitude of contraction was lower in E+ steers dosed with 10, 15, and 20 µg ERV/kg BW/d. A quadratic dose effect ($P = 0.012$) was seen for amplitude (amplitude of contraction = 10.19 – 1.66 × dose + 0.27 × dose^2). There was no difference between type of seed and seed × dose ($P = 0.137$ and 0.483, respectively) on frequency of contractions (Fig. 5B), but a dose effect ($P = 0.018$) was seen. A quadratic dose effect ($P = 0.005$) was seen on frequency of contraction (frequency = 2.72 – 0.3 × dose + 0.01 × dose^2), with lower values in E+ steers dosed with 10 and 15 µg ERV/kg BW/d.

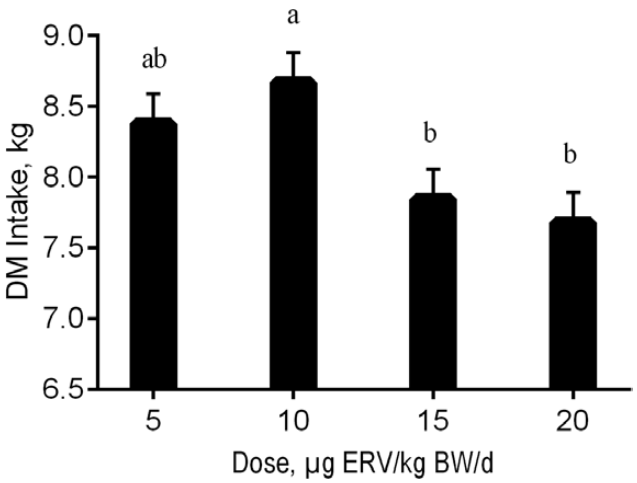

Figure 3. Mean results for daily DMI for the ergovaline dosage trial (Exp. 2) on steers fed ruminally dosed E– and E+ tall fescue seeds for 57 d. Means ± SEM, $n = 4$. The 0 treatment not shown because of pair feeding. Bars without a common superscript denotes difference ($P < 0.05$) between treatments, Dose: $P = 0.046$.

Experiment 3. Rumen Motility Measured by Wired Telemetry System and Reticuloruminal Fill in Steers Exposed to Intermediate Dosage of Ergovaline

During a period when steers were dosed with 15 µg ERV/kg BW/d for 21 d, there was no difference in DMI between treatment and control steers due to pair feeding (Table 5). A day effect was observed on DMI ($P < 0.001$) for the 21-d experiment, with the lowest intake on day 9 (data not shown).

During the days of motility collection, 24-h water intake was not different between E– and E+ treatments. Water intakes before and during motility collections (0800 to 1000 and 1000 to 1800 h) were lower in E– steers than in E+ steers (seed effect $P < 0.001$). Otherwise, there were no day and seed × day effects on water intake before and during motility collections. Serum prolactin was lower ($P = 0.011$) in E+ steers than in E– steers (seed effect $P = 0.011$). A day effect ($P = 0.0084$) was seen in serum prolactin with greater concentrations on day 22. There were no differences between E– and E+ treatments for all measures ($P > 0.05$) of rumen fill for Exp. 3.

Peak rumen contraction pressure was lower in E– steers than in E+ steers with seed and day effects ($P = 0.015$ and 0.001). Amplitude was lower in E+ steers with seed, day, and seed × day effects ($P = 0.001$, 0.001, and 0.048). Frequency of contraction was greater in E+ steers with seed ($P < 0.001$), day ($P < 0.001$), and seed × day effects ($P = 0.001$), and duration ($P < 0.001$) was also greater for E+ with seed and day effects ($P < 0.001$).

Experiment 4. Eating Patterns and Reticuloruminal Fill in Steers Exposed to Ergovaline

When steers were fed only once daily, rumen contents did not differ, but E+ steers had a tendency ($P = 0.07$) for rumen DM% to be higher than in E– steers (Table 6). On the other hand, there were no differences in rumen DM% between E– and E+ steers when they were fed 12 times a day. When steers were fed once a day, rumen DM content (kg) was numerically higher in E+ steers than E– steers, but there was no statistical difference ($P = 0.33$). The g rumen content DM per kg BW and g rumen DM per kg DMI were numerically higher in E+ steers than E– steers when steers fed once daily, but there were no statistical differences ($P = 0.33$ and 0.29, respectively) between E– and E+ steers. Rumen wet contents were not different across E– steers than E+ steers receiving feed 1 time or 12 times a day ($P = 0.96$ and 0.96, respectively).

Total amounts of feed offered to E– and E+ steers were the same because of pair feeding. However, the pattern of intake was slower in E+ steers than in E– steers (Fig. 6). When described

Table 3. Probabilities for orthogonal polynomial contrasts for the titrated-ergovaline dosage trial (Exp. 2) on rumen content analysis for steers treated with E+ and E– tall fescue seed for 57 d

	Contrasts on dose P-value					
	E–[1]			E+[2]		
Item	Linear	Quadratic	Cubic	Linear	Quadratic	Cubic
Rumen content, kg	0.309	0.877	0.498	0.011	0.053	0.141
Rumen content, DM %	0.244	0.867	0.234	0.470	0.937	0.277
Rumen DM contents, kg	0.062	0.939	0.097	0.176	0.234	0.592
Rumen wet contents, kg	0.440	0.865	0.661	0.016	0.086	0.129
g rumen content DM per kg BW	0.076	0.721	0.137	0.890	0.320	0.444
g rumen content DM per kg DMI	0.154	0.860	0.200	0.008	0.012	0.778

[1]E–, endophyte-free tall fescue seed.
[2]E+, endophyte-infected tall fescue seed; 5, 10, 15, and 20 µg ERV/kg BW/d.

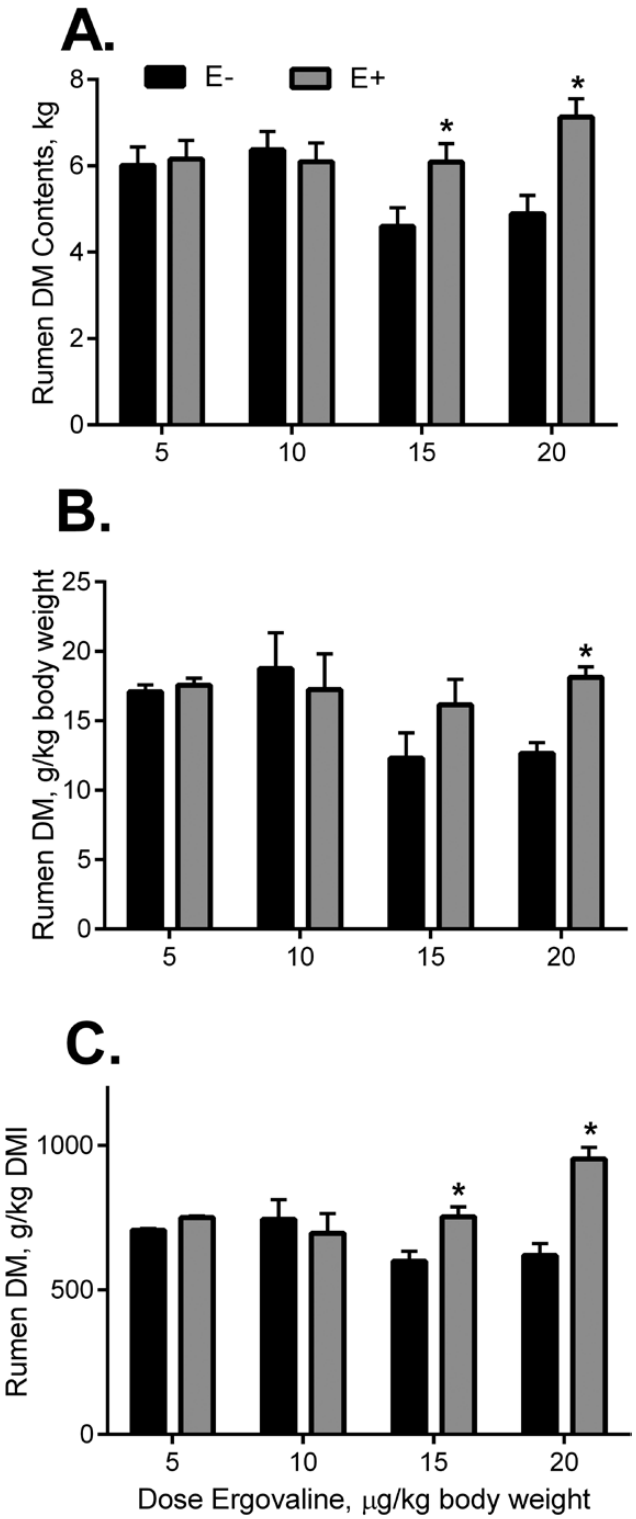

Figure 4. Mean results on ruminal contents for the ergovaline dosage trial (Exp. 2) of steers ruminally dosed E– and E+ tall fescue seeds for 57 d. Means ± SEM, $n = 4$. The 0 treatment not shown because of pair feeding. *Difference ($P < 0.05$) with dosage.

by an exponential function [Intake = Plateau * (1 – exp(–K*hour))] to estimate the fractional rate of intake, where plateau is the highest point or 100%, the constants were $K = 0.101$ and 0.659 for E+ and E–, respectively. Steers dosed with E– seed ate 63.8% of their feed in 1 h after feeding. Conversely, E+ steers required 9 h to consume more than 60% of their feed. The number of meals per day was higher ($P = 0.001$) in E+ steers than in E– steers when fed once per day (Table 7). On the other hand, meal duration and average meal size were not different between E– and E+ steers. The E– steers had higher water consumption ($P < 0.05$) than E+ steers when feeding 1 time a day (Fig. 7). Otherwise, average water consumption for 24 h was higher ($P < 0.05$) in E+ steers than in E– steers when steers were fed 12 times.

Discussion

A few studies have characterized ruminal dynamics in ruminants consuming emdophyte-infected diets. Goetsch et al. (1987) reported that passage rate of rumen fluid decreased with consumption of endophyte-infected hay when fed ad libitum and particulate passage rate was correlated with the DMI rate and was slower for endophyte-infected hay diets than control. However, when Hannah et al. (1990) demonstrated that with restricted-fed sheep, ruminal fluid volume was linearly decreased and fluid dilution rate and fluid outflow rate increased linearly with increasing concentrations of ergovaline consumed when compared with control. However, particulate passage rate was not altered by ergovaline, which could lead to an increased DM proportion of ruminal contents. These findings led to the hypothesis by Hannah et al. (1990) that increased ruminal contractions occur with exposure to ergovaline. In addition, Koontz et al. (2015) reported that particulate passage rate of cattle was decreased and liquid passage rate tended to decrease with endophyte-infected treatment resulting in greater DM percentage and DM weight of rumen contents. How ergovaline causes these reported changes in passage rates and rumen DM content is presently unknown. The experiments in the present study were designed to test the hypothesis that ergot alkaloids such as ergovaline alter rumen motility and (or) eating patterns.

The objective of the first study (Exp. 1) was to determine the effect of intraruminally dosed E– and E+ seeds in steers fed at a restricted intake (1.5 × NE_m) on rumen motility. Previous research Egert et al. (2014) demonstrated that a 14-d treatment period of 10 µg ERV/kg BW/d at thermoneutral conditions failed to induce fescue toxicosis; therefore, there was no difference in ruminal motility, fill, DM fill, and serum prolactin concentrations. Consequently, Exp. 1 was designed to again evaluate reticulorumen motility but provide a higher ERV dose to the steers, dosing at 20 µg ERV/kg BW/d. This dosage proved highly effective at inducing fescue toxicosis as steers dramatically reduced feed intake with increasing days of exposure; however, the ability to evaluate the impact of fescue toxicosis on rumen motility was hampered by these large changes in intake. Reduced DMI is a primary effect of cattle consuming endophyte-infected feed. Ergot alkaloids can act as agonists on serotongenic receptors (Dyer, 1993), and serotonin has been demonstrated to depress intake through increasing satiety signaling (Simansky, 1995). Several previous experiments also indicated that the exposure to ERV-depressed intake of steers (Goetsch et al., 1987; Aldrich et al., 1993; Koontz et al., 2012).

Another symptom of fescue toxicosis is reduced serum prolactin (Schillo et al., 1988; Aldrich et al., 1993; Koontz et al., 2012; Aiken et al., 2013; Foote et al., 2013). Likewise, in this study, serum prolactin concentrations were lower in E+ steers than in E– steers. This is an indication of a successful induction of fescue toxicosis across experiments.

Table 4. Mean results for the titrated-ergovaline dosage trial (Exp. 2) on reticuloruminal motility contraction variables measured for 57 d in E– and E+ tall fescue seed-treated steers

	Treatment			P-value			Contrasts on dose		
Item	E–[1]	E+[2]	SEM[3]	Seed	Dose	Seed × dose	Linear	Quadratic	Cubic
Amplitude, mm Hg	8.06	8.10	0.42	0.951	0.005	0.309	0.005	0.012	0.028
Frequency, contractions per min	1.67	1.23	0.07	0.137	0.018	0.483	0.111	0.005	0.313

[1]E–, endophyte-free tall fescue seed.
[2]E+, endophyte-infected tall fescue seed; 5, 10, 15, and 20 µg ERV/kg BW/d.
[3]SEM, standard error of the mean, $n = 4$ steers/treatment.

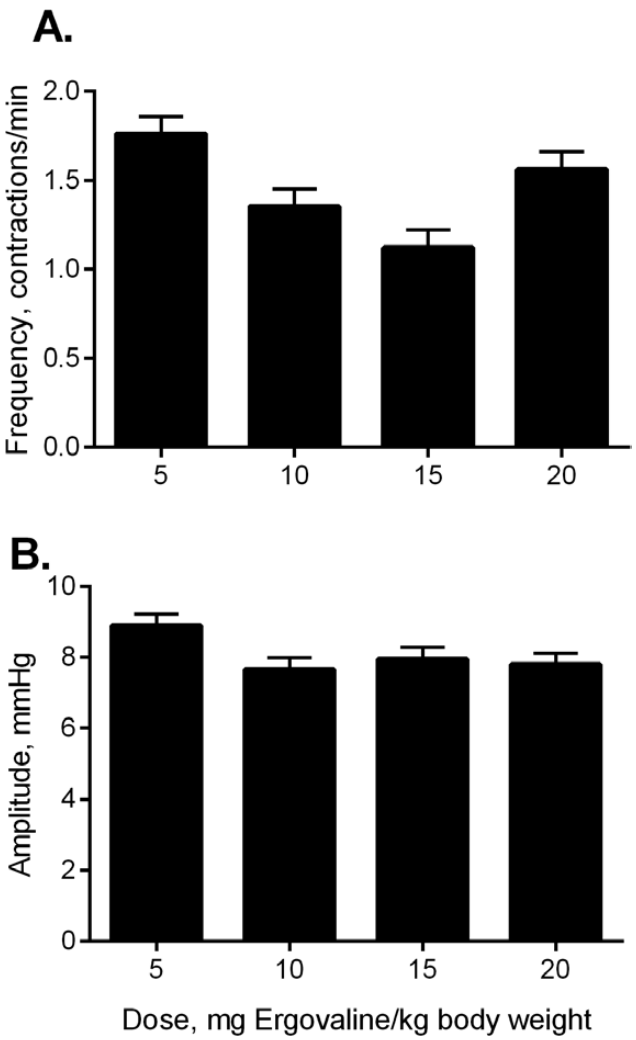

Figure 5. Mean results for the ergovaline dosage trial (Exp. 2) on reticuloruminal motility contraction variables measured for 57 d in E– and E+ tall fescue seed-treated steers. Means ± SEM, $n = 4$.

Ruckebusch (1988) reported that cattle showed 1.2, 2.0, and 1.1 contractions/min with amplitude of 18.2, 22.1, and 10.4 mm Hg during resting, while eating and throughout rumination, respectively. Cannulated cattle had 1.4, 2.0, and 1.1 contractions/min with amplitude of 5.9, 9.4, and 10.9 mm Hg while at rest, throughout eating, and during rumination, respectively (Mooney et al., 1971). The frequencies seen in this study (0.95 to 1.67 contractions/min) were similar to the range of those previously reported, but unlike Egert et al. (2014) who saw much higher frequencies (2.87 contractions/min). However, motility data for eating, resting, and rumination were not measured separately, rather daily averages were quantified.

Because of the dramatic decrease in DMI in Exp. 1, it was decided to examine motility responses at titrated ERV levels (0, 5, 10, 15, and 20 µg ERV/kg BW/d) in an attempt to more critically evaluate both fescue toxicosis and rumen motility. Steers were affected by E+ seed when fed at levels more than 15 µg ERV/kg BW/d with significantly higher rumen fill in E+ steers. This response was similar to previous work showing that ruminal DM content is increased with E+ treatments (Hannah et al., 1990; Foote et al., 2013; Kim et al., 2013; Koontz et al., 2015).

The amplitude and frequency of contractions were lower in E+ steers dosed with more than 10 µg ERV/kg BW/d. Previously, Egert et al. (2014) reported that rumen motility, rumen fill and DM of rumen contents were not affected by ERV dose when steers were fed 10 µg ERV/kg BW/d per day, suggesting this might be a critical level of ergovaline intake. In Exp. 4 of the present study, half of the experimental steers refused to eat E+ diets when fed 15 µg ERV/kg BW/d per day for 7 d. Thus, the trial was stopped and ERV dosing level was adjusted to 10 µg ERV/kg BW/d and restarted. There was a possibility that steers react to E+ seed more sensitively under the hot weather (Schmidt and Osborn, 1993), but in our study (Exp. 1 to 4), all steers were maintained indoors at 22 °C under thermoneutral conditions. Combined, these results suggest that there may be interactions with animal, ergovaline level, length of feeding, and so forth, making a single critical toxic dose was impossible to determine.

The impact of endophyte-infected seed on intake can be quite dramatic, which makes determining the impact on motility challenging. However, although there were definite measurable changes in motility, these changes were comparatively often small suggesting that motility may only be one factor affecting changes in ruminal fill. To test this, Exp. 4 was conducted to evaluate the eating patterns of steers fed E– and E+ tall fescue seed and simulating eating patterns of E+ steers to E– steers.

According to previous work (Hannah et al., 1990; Foote et al., 2013; Kim et al., 2013; Koontz et al., 2015), ruminal DM content is increased with E+ treatments compared with E– treatments, especially when intake is controlled, but to our knowledge, this is the first experiment describing eating patterns of steers fed E– and E+ seed. Goetsch et al. (1987) reported that steers fed endophyte-infected fescue showed different rates of digestion and passage, and it may induce different patterns of eating behavior and differences in daily intake. Conversely, the changes in eating pattern may drive rates of digestion and passage. Feeding behavior, such as time at feeder, time per meal, meal size, eating rate, number of meals, and visits to the feeder, is correlated with feed efficiency traits (Montanholi et al., 2010). These results indicate that ruminal DM content may be affected by eating patterns of steers.

Table 5. Mean results for the intermediate-ergovaline dosage trial (Exp. 3) on DMI, water intake, serum prolactin, and rumen motility contraction variables measured for 21 d in E– and E+ tall fescue seed-treated steers

	Treatment			P-value		
Item	E–[1]	E+[2]	SEM[3]	Seed	Day	Seed × day
DMI, kg	8.11	8.09	0.268	0.967	<0.001	0.999
24-h water intake, L	42.58	41.61	1.961	0.729	0.371	0.984
Water intake (0800 to 1000 h), L	8.29	3.32	1.024	0.001	0.731	0.183
Water intake (1000 to 1800 h), L	32.31	22.20	1.609	<0.001	0.250	0.494
Serum prolactin, ng/mL	107.80	29.05	20.212	0.011	0.008	0.549
Rumen content, kg	51.72	56.97	5.032	0.489	—	—
Rumen content, DM %	14.87	15.07	0.659	0.837	—	—
Rumen DM content, kg	7.67	8.57	0.712	0.409	—	—
Rumen wet content, kg	44.07	48.40	4.417	0.515	—	—
g Rumen content DM per kg BW	12.47	14.20	1.058	0.293	—	—
g rumen content DM per kg DMI	762.52	837.75	73.682	0.498	—	—
Peak, mm Hg	23.82	25.74	0.413	0.015	0.001	0.131
Amplitude, mm Hg	7.35	6.82	0.116	<0.001	0.002	0.048
Frequency, contractions/min	1.04	1.22	0.017	<0.001	<0.001	0.001
Durations, s	22.86	23.46	0.127	<0.001	0.001	0.276

[1]E–, endophyte-free tall fescue seed.
[2]E+, endophyte-infected tall fescue seed; 15 µg ERV/kg BW/d.
[3]SEM, standard error of the mean, $n = 4$ steers/treatment.

Table 6. Mean results for the trial to determine eating patterns (Exp. 4) on rumen content analysis for steers simulated with feed and E+ and E– tall fescue seed

	Feed 1 time a day[1]				Feed 12 times a day[2]			
Item	E–[3]	E+[4]	SEM[4]	P-value	E–	E+	SEM	P-value
Rumen content, kg	37.14	38.95	6.50	0.85	43.47	45.06	4.25	0.80
Rumen content, DM%	9.76	15.10	1.73	0.07	11.56	14.54	1.19	0.13
Rumen DM content, kg	3.81	6.02	1.43	0.33	5.19	6.56	1.01	0.37
Rumen wet content, kg	33.33	32.93	5.21	0.96	38.28	38.50	3.27	0.96
g rumen content DM per kg BW	6.75	10.62	2.52	0.33	9.24	11.52	1.76	0.39
g rumen DM per kg DMI	722.6	1,021.2	154.53	0.29	1,079.7	1,210.9	248.01	0.75

[1]Rumen evacuation occurred before feeding simulation; pair feeding; feeding 1 time at 0800 h.
[2]Rumen evacuation occurred after 7-d feeding simulation; feeding every 2 h, 12 times a day.
[3]E–, endophyte-free tall fescue seed.
[4]E+, endophyte-infected tall fescue seed; 10 µg ERV/kg BW/d.
[4]SEM, standard error of the means, n=4/treatment.

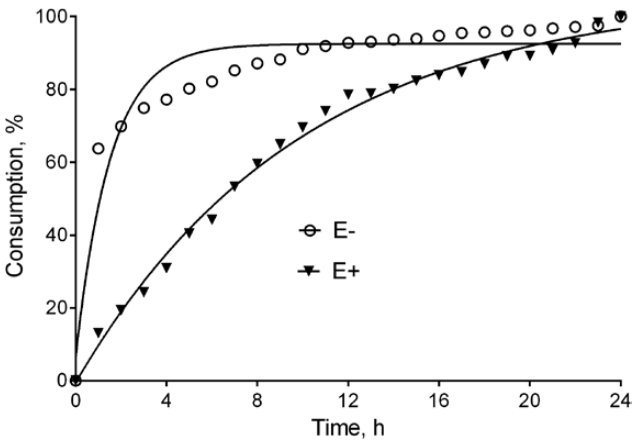

Figure 6. Feed consumption over 24 h for determining eating patterns of steers (Exp. 4) fed E– and E+ seeds. Means ± SEM, $n = 4$.

In this study (Exp. 4), feeding behaviors were described by feed consumption (kg/h), recorded by an automatic scale installed above feed troughs and then analyzed using a previously developed algorithm to identify a meal (Egert-McLean et al., 2019). This technique demonstrated that the number of meals per day was higher in E+-dosed steers than in E–-dosed steers, demonstrating that dosing ERV does affect feeding behavior of steers. Because E+ steers had more meals per day and their feed intake was the same because of pair feeding, average meal size of E+ steers was numerically smaller; however, there was no statistical difference.

The E– steers tended to eat their feed immediately compared with the E+ steers. According to the results of Exp. 4, E– steers consumed more than 60% of their meal in 1 h after receiving it. Conversely, the E+ steers need 9 h to consume more than 60% of their daily meal and generally took longer to consume their feed throughout the day. The assumption can be made that E– steers would consume more feed if DMI was not restricted to their E+ paired steer and that E+ steers reduced intake more

Table 7. Number of meals per a day, meal duration and average meal size for the trial to determine eating patterns (Exp. 4) of steers fed E– and E+ tall fescue seed

Item	E–[1]	E+[2]	SEM[3]	P-value
Number of meals per a day	4.38	7.81	0.67	0.001
Meal duration, min	18.78	20.87	3.29	0.656
Average meal size, kg	1.23	0.86	0.20	0.200

[1]E–, endophyte-free tall fescue seed.
[2]E+, endophyte-infected tall fescue seed; 10 µg ERV/kg BW/d.
[3]SEM, standard error of means, *n* = 4 steers/treatment.

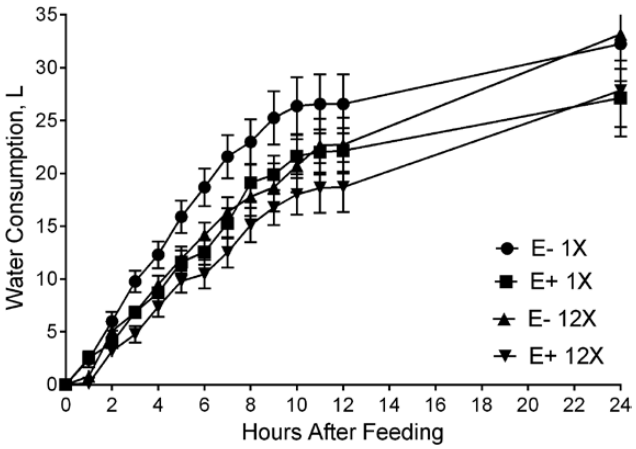

Figure 7. Water consumption over 24 h in the trial for determining eating patterns (Exp. 4) of steers fed E– and E+ seeds. Means ± SEM, *n* = 4.

than E– steers as time on treatment and concentration of ergot alkaloid increased. The reduced intake could be exacerbated by the increased ruminal fill, which increases distention and stimulates satiety centers (Ruckebusch, 1988).

In Exp. 4, the DM% of rumen contents tended to be higher in E+ steers than in E– steers when steers were fed 1 time a day. Because of the pair feeding, DMIs between E– and E+ steers were the same; thus, DM% of rumen contents tended to be higher in E+ steers than in E– steers due to the higher moisture content in rumen fill of E– steers. At this point, rumen DM content weight and g rumen DM per kg DMI should be higher in E+ steers than in E– steers. In this study, rumen DM content weight and g rumen DM per kg DMI were numerically higher in E+ steers than in E– steers, but not statistically different, because of the differences between the samples. This exemplifies the large differences in feeding behavior that occurred. However, when all steers received feed 12 times a day, rumen content (DM%) was no longer different between E– and E+ steers. Thus, the slower eating patterns of E+ steers may be one of the reasons that steers fed E+ showed a higher tendency of rumen content (DM%) than E– steers. E– steers had a longer period between completing their last meal before receiving the next-day ration. Also, when E– steers received feed 12 times a day, 24-h water consumption was lower than when E– steers fed 1 time a day (0 to 12 h and 12 to 24 h after feeding; Exp. 4). This result may also contribute to the observations that E– steers showed similar percent of rumen contents with E+ steers when they were fed 12 times a day.

Conclusions

This study was performed to evaluate how ruminally dosed ergot alkaloids affect ruminal motility, reticuloruminal fill, and eating patterns in steers. Data from these experiments indicate that feeding E+ seed does decrease frequency and amplitude of ruminal contractions. These changes along with slower eating patterns, and changes in the associated pattern of water intake for steers consuming E+ combine to increase ruminal fill and DM composition. Therefore, the changes in rumen fill associated with fescue toxicosis may contribute to the decreases in feed intake and the decreased productivity in ruminants consuming endophyte-infected forages.

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