Physiological and Behavioral Responses of Heifers that Graze Tall Fescue Infected by Wild-type or Novel Endophytes

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Abstract

Fescue toxicosis presents serious challenges and huge economic losses to the beef industry in the U.S. Replacing tall fescue (TF) infected by wild-type endophyte (WE) with TF infected by novel endophyte can eliminate this problem but adoption of this technology has been limited. We aimed at demonstrating the physiological and behavioral responses of heifers that grazed either WE or NE TF using relatively non-invasive techniques. Angus or Angus cross heifers (n = 24) were assigned to either WE or NE pastures for a 56-d grazing period during the summers of 2020 and 2021. Heifer ADG and hair retention scores were recorded once every 4 weeks and intravaginal temperatures were recorded for two consecutive days at this interval. Extremity temperatures were determined using thermographic imaging and hair was collected from the left rump for cortisol analysis. Animal behavior was detected using time-lapse trail cameras. The overall ADG of heifers that grazed NE was greater ($p = 0.0160$) compared to heifers that grazed WE in 2020, but not in 2021 ($p = 0.9623$). Hair retention was greater for heifers that grazed WE compared to heifers that grazed NE ($p = 0.0029$). Heifers that grazed WE TF had lower ($p \leq 0.0075$) temperatures at ears, tails, and hooves and 0.3-0.9 °C greater intravaginal temperatures than heifers that grazed NE, especially during daytime. Hair cortisol levels of heifers that grazed WE were greater ($p < 0.0001$) compared to heifers that grazed NE. From 1200h-1700h each day, heifers on WE pasture spent 1.5 more ($p = 0.0003$) hours loafing and 0.9 fewer ($p = 0.0402$) hours lying down than heifers on NE pastures. These results suggest that heat stress and other physiological changes in heifers grazing WE could be mitigated by renovating pastures with NE TF.

Introduction

Consumption of wild-type endophyte-infected tall fescue [*Lolium arundinaceum* (Schreb.) Darbysh.] by grazing animals is associated with a syndrome commonly known as fescue toxicosis. Fescue toxicosis is characterized by three common symptoms in livestock: fescue foot, fat necrosis, and summer slump (Strickland et al., 1993). Summer slump, a widespread condition associated with fescue toxicosis, occurs during warmer weather throughout the fescue belt. Common symptoms of summer slump include growth and retention of hair coats, reduced intake and weight gain, and reproductive losses along with various other physiological and behavioral changes (Strickland et al., 2009). Heat stress is commonly observed during times of high ambient temperature (>32 °C) and relative humidity (Spiers et al., 2005). Along with increasing insulation from greater hair growth, the toxic alkaloids in tall fescue are potent vasoconstrictors that constrict the blood vessels and reduce circulation to the body's extremities (Klotz et al., 2008). Scientists in the late 1990s discovered novel endophytes (NE) that produce very little to no ergot alkaloids, but which help maintain plant vigor and do not negatively impact animal performance (Bouton et al., 2002). Tall fescue infected with NE can reduce the effect of fescue toxicosis and improve livestock performance and is a valuable option for grassland renovation. Although several studies have compared the growth and reproductive performance of animals grazing WE and NE pastures less work has been done to compare the behavioral and heat stress responses of animals grazing these two pasture types. Thus, the objective of this study was to compare the behavioral and physiological responses of heifers grazing either NE or WE tall fescue using some novel and relatively less invasive measurement techniques such as hair cortisol, thermography (for extremity temperatures), intravaginal temperatures, and remote observations of in-field behavior.

Methods

The study was carried out during the summers of 2020 from mid-July to early September and in 2021 from late June to late August at Virginia Tech's Southwest Virginia Agricultural Research and Extension Center (SWAREC), Glade Spring, VA. This study utilized three WE tall fescue pastures (cv 'Kentucky-31') that were established about 25 years ago and three NE tall fescue pastures (cv 'Jesup MaxQ') that were established in 2007 (0.6-ha each). Twenty-four (24) fall-born Angus or Angus cross heifers 7-8 months old were stratified based on their body weight and hair coat color and randomly allocated to either of the two pasture types. Both pasture types were stocked with heifers for an 8-week grazing period each year. Pastures were rotationally stocked based on forage availability determined by visual observation of stand height. All the methods used in the study were approved by the Virginia Tech Institutional Animal Care And Use Committee under protocol # 20-017. Animal body weights (BW) were measured at the beginning (day 1), mid- (day 28), and end (day

56) of the study and were used to calculate average daily gain (ADG). The hair retention score of heifers was determined using a 5-point scale used by Gray et al. (2011). Thermal images of the body extremities (i.e., ear, front hooves, and tail tips) were captured using a FLIR T630SC thermal camera and were used to determine the external temperature of extremities. Intra-vaginal temperatures of heifers were collected with small temperature loggers (Star Oddi, Iceland) secured inside blank controlled internal drug release (CIDR) devices (Eazi-Breed, Zoetis, Parsipanny, NJ). These temperature loggers were placed into the vaginas of heifers twice for two consecutive days at 4-week intervals and collected temperature data every 10 minutes.

Hair samples were from an approximate 15 cm x 15 cm site on the rump region with an electric clipper at the beginning (day 0), mid (day 28), and end (day 56) of the grazing period. Hair cortisol extraction was performed according to the method described by Accorsi et al. (2008) using methanol. Blood samples were collected from the coccygeal vein and plasma was separated from blood samples by centrifugation at 3400×g at room temperature for 15 min. Cortisol was extracted from plasma samples using three, 5x volumes of ethyl ether. Sample tubes were dried overnight in a fume hood and reconstituted with ELISA buffer solution (Hinkle and Specker, 2003). Cortisol concentrations from hair and blood sample extractions were determined using a commercial test kit (Cortisol ELISA Kit Item No. 500360, Cayman Chemical, MI, USA), according to the manufacturer's instructions. For behavioral data, time-lapse images were collected using trail cameras set within each pasture to capture images at 1-minute intervals from 0800 to 2100h. For every image, the number of animals engaged in a given behavior (grazing, loafing, lying, drinking water, eating minerals) was recorded manually. The minutes spent in each behavior were summed and used to calculate the percent of time spent in each activity. Data were analyzed by mixed model analysis of variance test using PROC MIXED in SAS Studio, v9.4 (SAS Inst., Cary, NC). Year was used as a random effect in the model. Repeated measures analysis by period was used with a standard variance-covariance structure for the analysis of the data. LS-means and Tukey's adjusted differences were calculated. Differences were considered significant at $P < 0.05$ and trends at $P < 0.10$.

Results and Discussion

In 2020, the overall ADG was greater ($p = 0.0160$) for heifers that grazed NE tall fescue, with seasonal gains of 0.12 kg d^{-1} and 0.22 kg d^{-1} for WE and NE respectively (Table 1). However, in 2021, heifer ADG did not differ ($p = 0.9623$) by pasture treatment. Studies in the past have indicated that livestock grazing WE tall fescue have substantial production losses compared to non-toxic tall fescue (Parish et al., 2003; Klotz, 2015). Such differences were less pronounced for the heifers grazing WE fescue in this study. This is likely due to differences in weather patterns between the two years of the study as toxicosis severity is highly influenced by environmental conditions (Hemken et al., 1981). The mean THI in 2020 was 70.4 versus 70.0 in 2021. Also, the average THI exceeded the upper limit of the thermoneutral zone for cattle (72) for 15 days in 2020 but only 10 days in 2021. Also, heifers used for the study in 2021 were heavier than those used in 2020 (327 kg compared to 205 kg, respectively) which may have played some role in differential animal gain between years. Heifers in 2020 were used from a university herd located in the Shenandoah Valley, located several hours to the north of the study site, while heifers in 2021 were provided by a local producer. This difference in the origin of the heifers may also have contributed to differential animal gain between years. Heifers used in 2021 would likely be more adapted to the local conditions compared to those shipped in from the Shenandoah Valley in 2020. Long-distance transportation in 2020 to the study site may have exacerbated stress levels in heifers thus affecting their productivity. Decreased weight gain in heifers grazing WE tall fescue may be a result of reduced DMI due to heat stress induced by toxic alkaloids, given that lower DMI is a typical strategy for maintaining homeostasis when animals experience heat stress. The modest gain differences observed in this study might be due to its short duration (8 weeks), which may not have been sufficient to see a substantial treatment effect for this parameter. However, whether or not the environment was sufficiently stressful or the study was long enough to drive larger differences in gain, there were clear physiological and behavioral differences in heifers between treatments. Heifers that grazed NE fescue had a lower ($p = 0.0029$) overall hair retention score compared to heifers that grazed WE tall fescue. The follicular cycle in cattle is regulated by the prolactin hormone secreted by the pituitary gland. However, toxic alkaloid such as ergovaline within the tall fescue reduces the synthesis and secretion of prolactin (Aldrich et al., 1993) thus resulting in greater retention of winter hair.

Table 1 Average daily gain (kg d^{-1}) and hair retention score of heifers that grazed either toxic (WE) or novel (NE) endophyte-infected tall fescue during the summers of 2020 and 2021

Year	WE	NE	SE	WE	NE	SE
	ADG, kg d^{-1}			Hair Retention Score		
2020	0.12^{b}	0.22^{a*}	0.028	3.3^{a*}	2.8^{b}	0.15

2021	0.49	0.49	0.050	3.0 [a*]	2.5[b]	0.13
Average	0.31	0.36	0.031	3.1[a**]	2.7[b]	0.11

†Treatments: *WE* toxic endophyte-Infected tall fescue; *NE* novel endophyte-infected tall fescue
[ab]Means with different superscripts in a row differ (*p < 0.05, **p < 0.01)

Heifers that grazed WE tall fescue had cooler extremities ($p > 0.0075$) compared to heifers that grazed NE tall fescue (Table 2). Body temperatures at the extremities tend to be cooler in animals experiencing fescue toxicosis due to restricted blood flow, which in turn reduces animals' ability to dissipate body heat.(Rhodes et al., 1991; Aldrich et al., 1993). Various studies have reported decreased extremity temperature in animals in response to toxic ergot alkaloids consumption but to our knowledge, this is the first time that thermography, a relatively non-invasive technique, has been used to monitor this response. Overall, heifers that grazed WE tall fescue had 0.2-0.5 ºC hotter ($p \leq 0.02$) intravaginal temperatures between 1100h-1700h than heifers that grazed NE tall fescue (Fig 1).

Table 2 Extremity temperatures (ºC) of heifers that grazed either toxic (WE) or novel (NE) endophyte-infected tall fescue during the summers of 2020 and 2021

Year	WE	NE	SE	WE	NE	SE	WE	NE	SE
	Ear Skin Temperature, ºC			Hoof Surface Temperature, ºC			Tail Skin Temperature, ºC		
2020	29.5	30.6	0.45	24.9	26.3	0.66	26.0	25.5	0.35
2021	29.6[b]	31.8[a***]	0.25	27.8[b]	29.6 [a***]	0.21	27.2[b]	28.7 [a***]	0.25
Average	29.5[b]	30.2 [a***]	0.30	26.4[b]	28.0 [a**]	0.41	26.4[b]	27.4 [a**]	0.25

†Treatments: *WE* toxic endophyte-Infected tall fescue; *NE* novel endophyte-infected tall fescue
[ab]Means with different superscripts in a row differ (**p < 0.01. ***p<0.001)

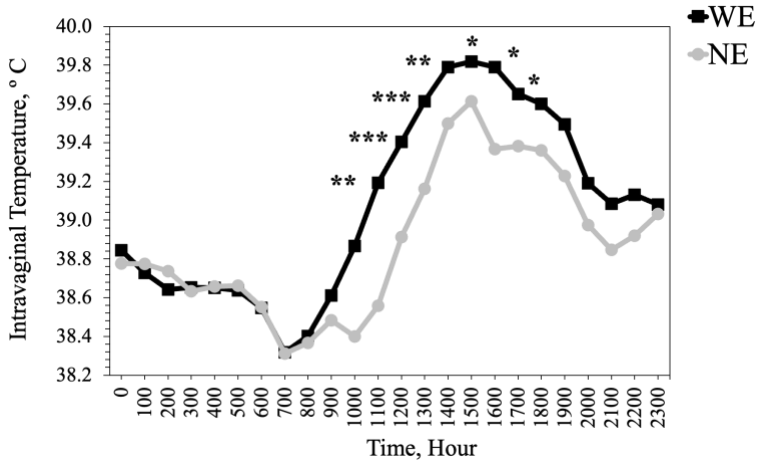

Figure 3 Comparison of mean vaginal temperatures (SE- 0.10) of heifers that grazed either toxic (WE) or novel endophyte (NE) infected tall fescue by the hour of the day. *Level of Significance- *P < 0.05, **P < 0.01, ***P < 0.001*

Hair cortisol levels were greater ($p < 0.0001$) for heifers that grazed WE pastures compared to heifers that grazed NE pastures across both summers (Table 3). The adrenal gland releases cortisol into the blood in response to a stressor and cortisol from the blood gets diffused into the hair at the follicle and accumulates over time. Thus, greater hair cortisol level reflects long-term chronic stress level in animals. The effect of vasoconstriction caused by the WE's toxic alkaloids is worsened by the retention of the winter hair coat and increased growth of the summer coat (Aiken et al., 2011). The increased insulative layer, coupled with the reduced ability to dissipate heat, causes a rise in core body temperature, and this stress results in a corresponding rise in cortisol levels.

Table 3 Plasma cortisol (ng ml^{-1}) and hair cortisol (pg mg^{-1}) concentration of heifers that grazed either toxic (WE) or novel (NE) endophyte-infected tall fescue during the summers of 2020 and 2021

Year	WE	NE	SE	WE	NE	SE
	Plasma Cortisol, ng ml^{-1}			Hair Cortisol, pg mg^{-1}		
2020	4.6[a*]	4.0[b]	0.19	8.1[a***]	6.3[b]	0.40
2021	10.3	9.2	1.31	5.0[a**]	3.2[b]	0.42
Average	7.5	6.6	1.32	6.6[a***]	4.7[b]	0.29

†Treatments: *WE* toxic endophyte-Infected tall fescue; *NE* novel endophyte-infected tall fescue
[ab]Means with different superscripts in a row differ (*p < 0.05, **p < 0.01, ***p < 0.001)

Heifers that grazed WE tall fescue spent less time lying and more time loafing especially during the afternoon hours (1200 - 1700) compared to heifers that grazed NE tall fescue (Table 4). This behavioral response of heifers is likely due to the effect of fescue toxicosis on the thermoregulatory ability of the heifers and the effects of ambient temperatures (Aldrich et al., 1993). Increased standing time is an adaptive mechanism of animals to limit heat gain from the ground surface and enhance heat loss through improved airflow to the body

surface and greater convective heat loss from the body (Ratnakaran et al., 2017). Thus, heifers on WE tall fescue likely spent more time standing as a strategy to reduce head load. The THI during the afternoon hours, when behavior data were recorded, was above the thermoneutral zone of cattle, thus exacerbating the effects of fescue toxicosis on these animals.

Table 4 Percent of time spent by heifers under different behavior categories on either toxic (WE) or novel (NE) endophyte-infected tall fescue pastures during the summer of 2020

Behavior Category	Morning (700-1200)			Midday (1200-1700)			Midday (1200-1700)			Overall		
	WE†	NE	SE	WE	NE	SE	WE	NE	SE	WE	NE	SE
Grazing (%)	30.0	22.0	2.98	19.3	31.4	4.80	60.9	58.5	6.76	36.7	37.3	1.83
Loafing(%)	44.2	42.0	9.79	63.4^{a***}	33.5^{b}	1.77	15.0	25.4	7.89	40.9	33.7	4.78
Lying (%)	25.1	35.9	9.20	17.2^{b}	34.9^{a*}	4.18	23.8	15.6	3.58	22.1	28.8	3.45
Drinking Water (%)	0.27	0.10	0.16	0.01	0.19	0.06	0.12	0.49	0.26	0.13	0.26	0.088
Eating Mineral (%)	0.36	0.02	0.05	0.08	0.00	0.049	0.21	0.05	0.15	0.22	0.02	0.049

†Treatments: *WE* toxic endophyte-Infected tall fescue; *NE* novel endophyte-infected tall fescue
[ab]Means with different superscripts in a row differ (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$)

Conclusions

Tall fescue is the predominant pasture forage in the southeastern U.S., but the wildtype endophyte within the plant produces toxins that act as a vasoconstrictor thus increasing heat stress and resulting in significant production losses in the beef industry. The relatively non-invasive techniques used here were effective for measuring the negative physiological and behavioral effects of fescue toxins. This study demonstrated both the increased vulnerability to heat stress and other physiological changes caused by consuming WE tall fescue and the improvements in animal welfare possible by converting toxic pastures to NE tall fescue.

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