Meta-analysis of endophyte-infected tall fescue effects on cattle growth rates

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ABSTRACT: The objective of this study was to quantitatively summarize literature reporting endophyte-infected (*Neotyphodium coenophialum*) tall fescue (*Festuca arundinacea*) effects on cattle ADG. This meta-analysis evaluated endophyte infection level, climate, and forage yield using a literature dataset of 138 treatments from 20 articles. Three infection level measurements were tested: endophyte infection as a percentage of infected tillers (E%); ergovaline concentration in ppb ([E]); and total ergot alkaloid concentration ([TEA]). Three types of climate variables were used: base values (temperature, humidity, and relative humidity), climate indices (heat index and temperature-heat index [THI]), and novel climate variables accounting for duration of climate effects. Mixed effect models, weighted by 1/SEM, including a random effect of study were built for each factorial combination of measurement method and climate variable group. Because many studies were missing SEM, two datasets were used: one containing only data with SEM reported and one that also included missing-SEM data. For the complete-SEM dataset (CSD), models were weighted by 1/SEM. In the missing-SEM dataset (MSD) the mean reported 1/SEM was assigned as the weight for all missing SEM treatments.

Although 18 initial models were created ($2 \times 3 \times 3$ factorial approach), the backward stepwise derivation resulted in models that included only endophyte infection level, suggesting a negative relationship between infection level and ADG. The CSD models predicted ADG to decrease 39 and 33 g/d with each increase of 100 ppb of [TEA] and [E], and by 39 g/d for each increase of 10% E%. In the MSD dataset, predicted ADG decreased by 39 and 33 g/d with each increase of 100 ppb of [TEA] and [E], and by 47 g/d for each increase of 10% E%. All relationships reported had $P < 0.05$. After visual inspection of the data, piecewise regression was used to identify an infection threshold (IT) of 60 ppb [E] and 11 E%, where the effect of infection level was constant on either side of the IT. The ADG was 40% and 49% greater for infection levels below the IT for [E] and E%, respectively. Across THI values in the analysis, ADG decreases ranged from 11.2% to 45.0% for cattle grazing endophyte-infected tall fescue compared to non-ergot alkaloid endophyte infected tall fescue. Pasture E%, [E], and [TEA] have a negative relationship with ADG in growing cattle, and increasing temperature decreases ADG when infection level is greater than the IT.

Key words: cattle, endophyte, ergovaline, fescue, growth, meta-analysis

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INTRODUCTION

Non-ergot alkaloid endophyte-infected (NEA) tall fescue is an alternative to toxic

wild-type endophyte infected tall fescue because it has greater stand persistence than endophyte-free tall fescue (EFF; Clay, 1988). Accordingly, cattle grazing NEA fescue have greater ADG than those grazing EFF (Parish et al., 2003; Nihsen et al., 2004). A challenge with adopting NEA tall fescue cultivar is the initial investment required to convert to novel cultivars; time required for transition

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is estimated at 3 to 7 yr (Bouton et al., 2002; Gunter and Beck, 2004; Beck et al., 2008). Without planning tools to evaluate this transition, evaluating economic viability is difficult. Quantitative cattle performance expectations on different cultivars is necessary for such planning.

Because of the large number of cultivars and the limited number of studies on each, it is more feasible to evaluate cattle responses to endophyte infection level. Three infection level metrics are reported: endophyte percentage (E%) measured as the proportion of infected tillers; concentrations of ergovaline ([E]); and total ergot alkaloid concentration ([TEA]). The literature on endophytes currently contains studies using E%, [E], and [TEA], and production expectations must also account for these variable reporting methods.

The objective of this study was to develop equations to describe performance of cattle grazing infected tall fescue pastures using meta-analysis of literature reporting on endophyte infection level and cattle ADG. Climate variables were investigated for effects on growth, and novel climate variables were created to better model the cyclic nature of climate's effect on endophyte growth. A secondary objective was to investigate the possibility of an infection threshold (IT), where infection effect differs in relation to IT. It was hypothesized that ADG would be decreased with increasing infection level and that other factors, such as climate, would exacerbate these effects.

MATERIALS AND METHODS

To properly assess the effects of grazing endophyte-infected tall fescue on stocker cattle ADG, a dataset was compiled and the papers were screened based on the inclusion and exclusion criteria detailed below. Climate variables not reported in papers were obtained from public weather station data repositories based on the dates and durations of the appropriate studies. Climate indices were calculated in an attempt to more succinctly describe climate variables. Novel parameters for the effect of study start date, temperature, and duration were created using sinusoidal curves to account for changes in environment over time. Initial models were designed using a $2 \times 3 \times 3$ factorial approach with factors being weighting strategy, climate variable, and measurement type. The 18 resulting models were derived using backward, stepwise elimination multiple regression and tested for significance. Models were compared using corrected Akaike information criterion (AICc) and estimated variance of study and error. Only models that resulted

in significant parameters are reported as results of the meta-analysis.

Data Collection

Data were collected from peer-reviewed published journal articles through a comprehensive literature search. Key words used to search for relevant articles were: fescue, endophyte, infected, ergot alkaloid, toxicosis, ergovaline, gain, and cattle. Subsequent titles were searched from the references of recovered articles, allowing an increased search space. To be included in the dataset, papers needed to be published before 2017 in English and include data for ADG using full live BW. Articles reporting the effects of tall fescue grazing on animals other than cattle were also excluded. Test animals that were fed an endophyte-infected seed concentrate were not considered for inclusion in the dataset because the focus of the study was on grazed tall fescue.

The complete dataset included 138 treatment means from 20 articles. Summary statistics for key variables reported within the dataset are provided in Table 1 and a listing of article citations is included in Supplementary Table 1. Of the 20 articles, six included growth data for heifers and cows instead of, or in addition to, data on steers. The growth curves of these animals would differ, but the addition of a random effect for trial should have accounted for this variation. All animals included were *Bos taurus* species with a combination of predominantly Angus and Angus crossbreeds.

Weighting Strategies

A challenge with meta-analysis is incomplete reporting of SEM. Often, papers that fail to report SEM are removed entirely from the dataset, and thus conceptually are given a weight of 0 during the fitting process. However, weighting these studies as 0 is likely inappropriate because they do have some value, it is just difficult to determine the exact value because the precision of the reported means is unknown. As an alternative to weighting the studies with a value of 0, studies were weighted equally to the average study in the dataset. Because this weighting approach is not conventional, two approaches were employed: the complete-SEM dataset (CSD), including only studies with reported SEM and the missing-SEM dataset (MSD), including all data with the mean SEM given to all incomplete data.

Data were weighted for $1/\text{SEM}$ to limit the weight of studies with very small trials and error. Optimal weighting using this method has been

Table 1. Summary statistics of the key variables in the study dataset, including missing-SEM data

Item	Na	Mean	Median	Minimum	Maximum
Studies	20				
ADG, kg/d	138	0.683	0.618	-0.340	2.32
Initial Weight, kg	70	256	247	220	437
Final Weight, kg	70	354	346	176	687
Forage Yield, kg/ha	33	3,020	2,850	1,350	8,980
Climate					
Max Temp, °C	131	28.0	26.8	12.7	43.3
Max Temp SD, °C	80	6.95	6.8	3.01	9.62
Average Temp, °C	135	18.4	20.7	2.83	36.0
Average Temp SD, °C	80	6.48	6.36	2.84	9.06
RH, %	127	63.7	60.2	52.1	77.0
RH, SD, %	80	16.3	16.1	8.42	21.1
THI	127	63.8	66.7	48.1	73.4
HI	127	64.1	68.7	41.3	77.6
Tall Fescue					
Endophyte, %	55	42.7	35.0	0.00	98.3
Ergovaline, ppb	44	196	31.5	0.00	1,210
Total Ergot	24	145	20.0	0.00	820
Alkaloids, ppb					

^aNumber of data points in missing-SEM dataset.

previously documented (Roman-Garcia et al., 2016; White et al., 2016) and works well with mixed models (St-Pierre, 2001). All papers included in the dataset were checked to determine whether the statistical analysis used a fixed or mixed effect model. The weighting factor was calculated by dividing the reciprocal SEM by the mean reciprocal SEM within analysis type. Dividing by the mean reciprocal SEM normalized the weighting factors to 1 irrespective of analysis type, effectively standardizing the weights across fixed effect or mixed models (White et al., 2015; Roman-Garcia et al., 2016). In studies that reported SEM as less than one-fourth the mean SEM, the SEM was set to one-fourth of the mean SEM across all studies to prevent over weighting (Firkins et al., 2001; Roman-Garcia et al., 2016; White et al., 2016). The curtailing of SEM resulted in 5.2% and 3.6% of errors being trimmed for CSD and MSD, respectively. The SEM trimming was conducted separately for mixed and fixed effect models, because mixed-models had greater SEM. The result of this cleaning was weighting factors equivalent to the reciprocal SEM without bias for statistical method, without overweighting extremely precise studies.

Variables to Represent Climate

Raw climate variables of interest within this study included mean, maximum, and standard deviation of temperature by month. Mean

humidity was also considered. Climate data were gathered for each study from the National Centers for Environmental Information's local climatological database (National Climatic Data Center [NCDC]). Data were downloaded from the database and mean temperatures, dew points, and maximum temperatures for the duration of the studies were recorded. The relative humidity (RH), which the sourced weather data did not supply, was calculated using the formula:

$$RH, \% = 100 \times e^{\frac{17.625 \times TD}{243.04 \times TD}} / e^{\frac{17.625 \times T}{243.04 \times T}}$$

where T is Temperature in degrees Celsius and TD is the Dew Point (Alduchov and Eskridge, 1996).

As a means to potentially reduce complexity in the models, and better describe climate effects, various indices were employed to represent the effect of climate on ADG. Because previous work has suggested cattle consuming endophyte infected tall fescue are hypersensitive to heat, specific focus was placed on variables that would reflect heat stress, including: heat index (HI) and temperature-humidity index (THI).

The HI was calculated as:

$$HI = 0.5 \times \{T + 61.0 + [(T - 68.0 \times 1.2)] + (RH \times 0.094)\}$$

where T is Temperature in degrees Celsius and RH is Relative Humidity as a percentage (Rothfusz and Headquarters, 1990).

A metric combining temperature and humidity is often employed to more accurately represent the heat load on the animal. The most common technique, used in The Livestock Weather Safety Index (LCI, 1970), is the THI. The THI was calculated using the equation:

$$THI = 0.8 \times T + \left[\frac{RH}{100} + (T - 14) \right] + 46.4$$

where T is Temperature in degrees Celsius and RH is Relative Humidity as a percentage. The THI has been shown to effectively indicate heat stress in cattle, and continues to be refined with more recent adjustments for wind speed and solar radiation (Mader et al., 2006). Although wind speed and radiation have been shown to influence heat stress, these data were not available in the current study. However, Mader et al. (2006) suggest that THI is an adequate representation of thermal load with or without adjustment for wind speed and radiation.

It has been documented that increased endophytic infection leads to greater vasoconstriction, making it more difficult for animals to dissipate heat (Rhodes et al., 1991; Oliver et al., 1998). Research has also shown that E% and [E] change throughout the year relative to maximum infection potential, regardless of location (Ju et al., 2006). Given the concurrent change in temperature and infection percentage, it was hypothesized that a variable representing climate impacts on tall fescue ADG responses should also represent the temporal behavior of stand infection. Table 2 includes data from Ju et al. (2006), which was used to derive a curve to describe the effect of time of year on endophyte levels. A curve for E% and [E] effects as a percentage of max infection level were derived using nonlinear least-squares regression and the following formula:

$$\text{Effect}\% = A \times \sin(2\pi ft + \phi) + \beta$$

where A is Amplitude, f is Frequency, t is Time in months, ϕ is the Phase and β is the y-intercept ($P < 0.001$ for all parameters). A , f , ϕ and β were found to be 0.331, 0.082, 2.291, and 0.645, respectively. By fitting sinusoidal curves to E% and [E] the effects of infection level over time were quantified. Trial duration was accounted for by integrating over the curve in 1/30th-mo intervals from the start date of the trial to determine the mean effect of infection level experienced by the animals on any

specific trial. The equation for average endophyte level over a given duration (AEL) was:

$$\text{AEL} = \frac{1}{\text{duration}} \sum_{i=\text{start}}^{\text{start}+\text{duration}} \text{Effect}\%_i$$

where *start* is the start month of the trial and *duration* is the length of the trial as a fraction of a month. This resulted in a number that, in theory, more accurately described the combined thermal and endophyte consumption effects experienced within each treatment group.

Infection Threshold

The IT, defined as the threshold to produce clinical tall fescue toxicosis, has been reported as 300 to 750 pbb [E] (Hovermale and Craig, 2001; Tor-Agbidye et al., 2001; Craig et al., 2014). Along with the three common measurement methods previously described: E%, [E], and [TEA], an IT was derived by piecewise regression to analyze the effect of splitting the response surface. This approach fits one slope to infection level greater than a threshold and a different slope below the threshold. The threshold value was identified by iteratively testing models over the sample space of each infection level measurement to determine the optimal threshold. The IT value was chosen based on the infection level identified to generate the smallest AICc (Hurvich and Tsai, 1989). Once a threshold was identified, forward stepwise regression was used to test for significance of climate variables.

Model Derivation Procedure

All models were derived using the lmer (Bates et al., 2017) function in R version 3.1.0. (R Core Team, 2017) using mixed-effect weighted regression including a random intercept for study. Growing cattle ADG was used as the response variable for all models. The inclusion of a random study effect should control for mean differences in BW across studies and limit the potential confounding effect of BW on ADG. All explanatory variables are summarized in Table 1. For the set of 18 factorial models, models were refined through backward stepwise elimination multiple regression as described in Roman-Garcia et al. (2016) and White et al. (2016). The variable with the greatest nonsignificant P -value ($P > 0.05$) was iteratively eliminated from the model unless the term was a linear term with a significant quadratic effect ($P \leq 0.05$). The piecewise models were derived as previously described. After the threshold infection percentage was identified,

Table 2. Data for ergovaline concentrations^a and average temperature by month from the missing-SEM dataset

Month	Endob	EndoMax, %c	Tempd	TempMax, %e
January	0.57	0.28	3.77	0.14
February	0.77	0.37	5.20	0.20
March	0.66	0.32	10.2	0.38
April	0.93	0.45	15.1	0.57
May	1.25	0.60	20.2	0.76
June	1.52	0.73	24.5	0.92
July	1.93	0.93	26.2	0.98
August	1.80	0.87	26.6	1.00
September	1.87	0.90	22.0	0.83
October	2.07	1.00	16.3	0.61
November	1.61	0.78	10.5	0.39
December	1.16	0.56	12.0	0.45

^aErgovaline data adapted from Ju et al., 2006

^bTotal endophyte concentration, mg/g.

^cErgovaline as a decimal percentage of the maximum concentration recorded.

^dTemperature, °C.

^eTemperature percentage as a decimal of maximum temperature recorded.

forward stepwise regression was used to test for parameter significance.

Final models were also checked to ensure all variance inflation factors (VIF) were acceptable. The VIF measures the severity of the multicollinearity in a regression and the resulting severity of the inflation of variance due to this collinearity in the parameter estimations. The square root of the VIF indicates the inflation of the variance of the parameter estimate compared with an ideal scenario of no collinearity. This study used a VIF cutoff of $VIF < 10$ for linear factors not involved in interactions or quadratic terms, meaning the variance due to collinearity was one factor greater than that of a regression with no collinearity in parameter estimates. The cutoff for quadratic terms, interaction terms and linear terms involved in either quadratic or interaction terms was $VIF < 100$. These cutoffs are in line with current research practices, although no clear rules have been established (Roman-Garcia et al., 2016).

Evaluating Model Performance

Models were evaluated based on AICc and root estimated variance due to error (i.e., the estimated variance for error) and study; $\hat{\sigma}_e$ and $\hat{\sigma}_s$, respectively. Both $\hat{\sigma}_e$ and $\hat{\sigma}_s$ are expressed as a percentage of the dependent variable mean. The AICc was the predominant indicator of a more accurate model because all models were derived from the same size datasets using the same response variable. The reporting of RMSE was avoided because of the inclusion of a random effect of study. When a random study effect is included and models are chosen based on RMSE, the models perform poorly on

new data with different studies because they underestimate error (Boerman et al., 2015). All variables were assessed for simple correlation to evaluate collinearity. Residual plots used data adjusted for the random effect of study, and the linear regressions were weighted for the SEM to check for patterns in the data. Slope and mean bias as a percentage of the dependent variable mean were recorded and any model displaying a significant bias was adjusted or removed. When models were comparable, the one with more observations was deemed more desirable.

RESULTS AND DISCUSSION

Equation Descriptions

A set of linear models was derived using a $2 \times 3 \times 3$ factorial approach with factors for weighting strategy, climate variable, and measurement type. The final equations for each of the 18 factor combinations can be viewed in Table 3. When different factorial combinations lead to the same equation, the resulting equation was only listed once.

Average Daily Gain Responses

After backward elimination, infection level was the only significant variable in all tested models. The three equations using E%, [E], and [TEA] were considered the basis of comparison for all other models and can be found in Table 3. Each of the three measurement methods had significant ($P < 0.05$), negative relationships with ADG. Using the derived equations from the CSD: a 10%

Table 3. Parameter estimates in models of ADG using either the complete-SEM dataset or including the missing-SEM data

Item	Complete-SEM Dataset			Missing-SEM Dataset		
	E%	[E]	[TEA]	E%	[E]	[TEA]
Equation no.	1	2	3	4	5	6
Intercept	0.7342	0.7443	0.5969	0.7985	0.7302	0.7834
E%	-3.885×10^{-3}			-4.647×10^{-3}		
[E]		-3.254×10^{-4}			-3.259×10^{-4}	
[TEA]			-3.934×10^{-4}			-3.912×10^{-4}
Fit statistics						
<i>n</i>	32	41	22	55	44	24
AICc	5.09	-33.4	-4.70	68.3	-34.1	1.52
$\hat{\sigma}_s^a$	0.037	0.120	0.274	0.440	0.103	0.555
$\hat{\sigma}_e^b$	0.115	0.106	0.076	0.242	0.110	0.074

Models were further divided by method used to measure infection level.

^aSquare root of the estimated variance associated with study.

^bSquare root of the estimated variance associate with residual error.

increase in E%, a 100 ppb increase in [E] and a 100 ppb increase in [TEA] was associated with a decrease in ADG of 38 g (5.3%), 33 g (4.4%), and 39 g (6.6%) per day, respectively. Barker et al. (2009) noted that a rule of thumb for E% is a loss of 45 g/d for each 10% increase in E%, which is marginally greater but generally in line with the CSD estimates.

Measurement Methods

Equations utilized one of the three base methods for measuring endophyte levels in tall fescue: [E] in parts per billion, E% and [TEA] in parts per billion. From 1983 to 1993, the popular method for measuring endophyte levels involved taking samples from tillers in the field and inspecting the samples for the presence of endophytes. The results of this microscopy work were reported as infected tillers as a percentage of total samples taken (E%). This method was used until Rottinghaus et al. (2001) developed a method of using High-Performance Liquid Chromatography (HPLC) to measure [E], the most abundant toxic alkaloid produced by the endophytes (Rottinghaus et al., 1991). This HPLC measurement remains the most common measurement method in research on endophyte-infected tall fescue due in part to the recent creation of non-ergot alkaloid endophyte strains, which do not produce ergovaline.

Non-ergot alkaloid endophytes present in tall fescue still appear using microscopy, but do not create the harmful ergot alkaloids that are toxic to cattle. As a result, novel cultivars cannot be reliably distinguished from endophyte infected tall fescue when using the E% method. Because of the growth in popularity and interest in endophyte-infected tall fescue that does not produce ergot alkaloids, HPLC is distinctly more effective at properly quantifying the infection level key to decreased ADG in cattle. Using E% as the measurement method (equation 1) yielded the greatest slope and mean error as a percentage of the mean SE in the CSD. In terms of variation, the E% equation parameter estimate CV was greatest among all equations, with CV of 130%, 93%, and 77% for equations 1, 2, and 3, respectively. When coupled with inability to differentiate toxic from novel, nontoxic cultivars, these observations suggest E% is a poor indicator of ADG.

The [E] has been cited as making up between 85% to 97% of [TEA] in tall fescue (Lyons et al., 1986). Using the models derived herein, the proportion of ADG loss associated with [E] and [TEA] can be calculated. This calculation was

done using the following simple arithmetic and equations 2 and 3.

$$ADG, kg/d = ([E] \times -3.254 \times 10^{-4}) + 0.7443$$

$$ADG, kg/d = ([TEA] \times -3.934 \times 10^{-4}) + 0.5969$$

$$[E] = [TEA] \times 1.209 + 453.0$$

Using this equation, the effect of any given [TEA] in terms of an [E] can be derived. Taking into consideration the almost identical slopes of equations 7 and 8, the difference in the effect of an increase in [TEA] vs. [E] is approximately 20%. This suggests that ergovaline accounts for at most 80% of the effect on ADG of the [TEA] produced by a given tall fescue cultivar; 80% reflects a maximum because there could be effects common between [E] and [TEA] such as shade, water access, mineral supplementation, or some other factor. Note that this percentage is different than the aforementioned 85% to 97% for the proportion of ergovaline in the [TEA]. Although [E] makes up at most 80% of the effect on ADG, the remaining 3% to 15% of ergot alkaloids, along with factors like genetics or pasture management, are likely responsible for the other 20% of the ADG response.

Because there is a linearly correlated relationship between the effect of [E] and [TEA] ($r \approx 1$, $P < 0.001$), measuring one should provide a good proxy for the other at any concentration. Although the difference in slope between the two equations was negligible, the proportion of effect attributed to [E] may decrease at high concentrations; [E] makes up about 65% of the total effect of [TEA] at 800 ppb.

Climate Variables

Although variables were systematically added back into the final models to identify the best combination, no climate variables were identified as significant for the three measurement methods using the CDS ($P > 0.05$). There are several reasons why the climate variables did not appear to have a significant effect on ADG. In the mixed effects models, publication was modeled as a random effect to account for study-to-study variation. Because of the small number of trials included for each measurement type, three, five, and six publications for [E], E% and [TEA] in the CSD and four, eight, and seven publications for the MSD, this random effect may explain most of the climate variation. In the 14

different publications included in the CSD, states in which trials were performed were Arkansas, Oregon, Kentucky, Georgia, Tennessee, Alabama, Oklahoma, Louisiana, and North Carolina. The state with the most studies in the dataset was Georgia, with five different treatment groups. By isolating these points, the resulting box plot (Figure 1) illustrates a relationship between THI and ADG. When only these data were analyzed, a 75% decrease in ADG was observed as THI moved up approximately 3.5 units ($P < 0.01$). This relationship may be an overestimate due to limited sample size; however, this illustrates the possibility of climate variables having significance that was not apparent in equations 1, 2, and 3.

As noted previously, research has shown a change in infection rates over the year, with greater infection levels occurring in the warmer months (Ju et al., 2006). The fact that both infection levels and temperature rise in parallel could be another reason why a significant interaction between infection level and temperature was not detected. In the models, some of the effects of increasing infection rates may be attributed to rises in climate variables that compound on an animal's ability to dissipate heat. Within these data it is likely that infection level represents both the actual infection level of the forage but also the season in which the measurements were collected.

A duration by starting month interaction variable was included at the beginning of backwards regression in each model using the raw climate data factor, with the effect being removed due to non-significance each time. The P -value of duration by starting month when combined with each measurement method alone was only significant ($P = 0.014$) for [TEA]; however, the AICc value was greater in this model and was considered inferior when compared to predictions using [TEA] alone. For models using [E] and E%, adding a duration by starting month interaction variable either showed a tendency towards significance or was non-significant ($P = 0.069$ and 0.8114 , respectively) in the

Figure 1. Boxplot of relationship between THI and ADG in all Georgia data. Dataset was comprised of all studies done in Georgia within the dataset to determine a relationship between THI and ADG not observed in the models derived for ADG.

CSD. This result could be due to the small sample size of starting months.

A reason for failure to identify significant climate effects in most models might be that THI and HI were not within ranges typically considered to be severe. Because THI and HI were calculated using the average temperatures provided, the indices may not have properly captured times of heat stress that may have occurred intermittently during the trials. Eigenburg et al. (2005) cites a maximum threshold THI of 74 for “normal” conditions; there were no trials with a calculated THI greater than 73. Although THI was assumed using average temperatures and RH would be a good proxy for severity of heat stress experienced, this may not have been the case in the current dataset.

The research goal was to utilize previous data to extrapolate possible climate and infection curves, then use those curves to better account for the average effect experienced by the animal using integration to account for duration of study. The CSD showed a significant ($P < 0.001$) negative correlation between duration of the studies included and the starting month (Table 4), meaning that longer studies tended to start earlier in the year and shorter studies at the end of the year. This makes sense from a logical standpoint because the prime grazing season is predominantly in the spring and summer. However, the correlation between duration and starting date made it difficult to test AEL.

Pairwise Regression Model Performance

Studies have identified an [E] threshold value needed to produce clinical signs of toxicity. The range of suggested IT was large, but these studies suggest a differing effect of infection greater and less than a threshold (Hovermale and Craig, 2001; Tor-Agbidye et al., 2001; Craig et al., 2015). To evaluate whether this relationship existed in the current data, piecewise regression was used to test models with two linear response surfaces based on IT. Previous studies have cited thresholds for clinical tall fescue toxicosis (Hovermale and Craig, 2001; Tor-Agbidye et al., 2001; Craig et al., 2015); however, these thresholds have not been derived based on quantitative summary of the available literature. In this analysis, IT models were only derived for [E] and E% due to the small sample size of the [TEA] data in the CSD and MSD relative to any threshold. The IT for [E] was found to be 60 ppb and 11% for E%. Using the IT, infection levels less than the IT resulted in a 40% and 49% increase in ADG for [E] and E%, respectively. The AICc graphs and visuals

Table 4. Correlation table for variables reported in studies

	Dur	Mon	RH	RH SD	Max.T	Max.T SD	Avg.T	Avg.T SD	ADG	Forage	HI	THI	[TEA]	[E]	E%	AEL
Dur	1.00															
Mon	-0.22 ^a	1.00														
RH	0.52	0.02	1.00													
RH SD	-0.30 ^a	0.59 ^a	-0.32 ^a	1.00												
Max.T	0.62	-0.16 ^b	0.83	-0.88	1.00											
Max.T SD	0.05	0.05	0.11	0.52 ^a	-0.59 ^a	1.00										
Avg.T	0.23 ^a	-0.25 ^a	0.49 ^a	-0.88	0.70	-0.60 ^a	1.00									
Avg.T SD	0.08	-0.06	0.21 ^b	0.39 ^a	-0.43 ^a	0.98	-0.44 ^a	1.00								
ADG	0.14 ^b	0.07	0.03	-0.44 ^a	0.01	-0.16	0.26 ^a	-0.11	1.00							
Forage	0.52 ^a	-0.16	-0.10	-0.72 ^a	0.06	0.09	0.00	0.02	0.02	1.00						
HI	0.39 ^a	-0.30 ^a	0.52	-0.88	0.72	-0.59 ^a	1.00	-0.44 ^a	0.26 ^a	0.00	1.00					
THI	0.41 ^a	-0.27 ^a	0.54	-0.87	0.73	-0.60 ^a	1.00	-0.46 ^a	0.27 ^a	-0.01	1.00	1.00				
[TEA]	0.00	-0.02	0.05	0.20	-0.09	0.00	-0.14	0.01	-0.46 ^a	1.00	0.20	0.19	1.00			
[E]	-0.07	0.01	0.12	-0.04	0.07	-0.12	0.07	-0.07	-0.57 ^a	0.05	0.07	0.07	NA	1.00		
E%	0.16	0.13	0.22	NA	0.23	NA	0.21	NA	-0.32 ^a	NA	0.23	0.23	NA	NA	1.00	
AEL	0.20 ^a	-0.57	0.17 ^b	-0.82	0.45 ^a	-0.55 ^a	0.54	-0.45 ^a	-0.14	0.48 ^a	0.47 ^a	0.45 ^a	0.03	0.01	0.03	1.00

Dur = Duration; Mon = Starting Month; RH SD = standard deviation of the RH; Max.T = Average Maximum Temperature, °C; Max.T SD = standard deviation of Max.T; Avg.T = Average Temperature, °C; Avg.T SD = standard deviation of Avg.T; Forage = Forage Yield.

^a $P \leq 0.05$.

^b $0.05 < P < 0.10$.

of the gaps in data are shown in Figure 2 and suggest that infection levels do not appear uniformly in the literature, but rather in discrete ranges.

Results of the IT models are shown in Table 5. For the [E] IT, a negative relationship was significant for the infected by average temperature interaction variable. The ADG decreased as temperature increased when the tall fescue infection level was greater than the IT. This is logical due to temperature exacerbating the effects of heat stress caused by cattle grazing infected tall fescue. The fact that temperature alone did not have a negative relationship with ADG is logical considering that increased temperature would indicate better growing conditions for forages, leading to greater ADG in the absence of infected tall fescue (lower than the IT). Despite the increase in predictor variables, the AICc value for the infection by average temperature model was lower for equation 2 using the same data. Using THI as the climate variable in place of average temperature also yielded significant results and a negative relationship between the infected by THI interaction variable (Table 5).

The use of a AEL variable to account for duration of study improved the results of the IT model, with the intercept, infected, AEL, and AEL by infected interaction variable all showing significance ($P < 0.05$). As with the raw climate indices, the interaction of AEL by infected had a negative relationship with ADG. As cattle graze infected tall fescue in times of greater infection levels, ADG decreases. In times where AEL would be high but the pastures are not infected, ADG increases.

Weighting Strategies

In the analysis of the MSD, a larger dataset was utilized by including data that did not report SEM of ADG responses. The value and significance of parameter estimates derived from the CSD and MSD datasets differed (Table 3). At this point, it is unclear whether these inconsistencies are caused by the weighting procedure (filling in missing SEM as equal to the mean SEM) or by the added data revealing new relationships. Future work on synthetic data evaluating different ways to handle missing SEM data are needed to better understand the best way to deal with this data challenge.

Missing-SEM data.

Results for models derived using the MSD are listed in Table 3. The estimation for ADG with no infection (intercept) changed +8.8%, -1.9%, and +31.2% from the estimations using the CSD for E%, [E], and [TEA], respectively. Changes in slopes for E%, [E], and [TEA] were +19.6, +0.2, and -0.6% compared to the CSD equations. Both the intercept and parameter estimations increased from equations 3 to 6, likely because the mean ADG within the MSD dataset was greater than the CSD dataset (0.6771 and 0.5650 kg/d, respectively).

Implications

Total endophyte concentration has been shown to increase starting in the spring, with lower concentrations in the winter (Ju et al., 2006). A curve

Figure 2. (A,B) AICc values associated with pairwise regression models built at an infection threshold (IT). The optimal IT was selected as the first point at which the AICc value reached its minimum. (C,D) Plots of infection measurement method, illustrating the sparsity of intermediate level infection data.

for [E] was created using year-long measurements of endophyte concentration because it was assumed that the fraction of ergot-alkaloid producing endophytes is proportional to total endophyte concentration throughout the year, which is reasonable considering E% fluctuates over a year in a cyclic manner as well. Studies have reported cyclic changes in [E] coinciding with head emergence and seed development in perennial ryegrass, and also suggest the highest [E] occurs during peak host

Table 5. Parameter estimates for models of ADG using the complete-SEM dataset with thresholds derived from E% and [E] separately¹

Item	Infection-only equations		Climate-included equations	
	E%	[E]	E%	[E]
Equation no.	15	16	17	18
Intercept	0.745	0.754	1.017	-0.172
E% Infected	-0.269		-0.226	
[E] Infected		-0.249		0.882
THI				0.016
AEL			-0.004	
[E] Infected × THI				-0.020
Fit statistics				
<i>n</i>	32	41	32	41
AICc	-3.5	-39.4	2.56	-50.3
$\hat{\sigma}_s^a$	<0.001	0.098	<0.001	0.041
$\hat{\sigma}_e^b$	0.117	0.114	0.104	0.078

^aSquare root of the estimated variance associated with study.

^bSquare root of the estimated variance associate with residual error.

growth in the summer (Reed et al. 2011). The values in Table 2 were used to calculate a curve for percent [E] of maximum exhibited in the year for each day of the year. For example, on March 1, [E] in a field will be 34% of the year's potential maximum [E]. Using three levels of field infection, 0, 100, and 300 ppb [E], differences in cumulative growth were calculated for cattle born during spring and fall calving seasons. Cattle BW gain was 33% and 19% greater in the 4-mo period from October through January for cattle on 0 ppb [E] fields compared to 300 and 100 ppb, respectively. From June through September, BW gain was 128% greater for cattle on 0 ppb [E] fields compared to both 300 and 100 ppb. A 0 ppb [E] field compared to a 300 ppb [E] results in an extra 20 and 65 kg BW gain in the fall and spring calving seasons, respectively. The cumulative BW gain over 1 yr, October through January, and June through September are graphed in Figure 3. Value per kg of BW gain was calculated using Missouri purchase prices from 2012 to 2017 of 249-kg BW, medium-framed, number 1 steers in September for fall grazing and February for spring grazing. The values calculated were \$1.27/kg and \$1.21/kg in U.S. dollars for fall and spring purchase prices, the growth difference among these systems would give the 0 ppb pasture a benefit of \$25 and \$79 per head per season. These values are considerable, but very much aligned with the data compiled. In two studies that reported ADG for cattle grazing tall fescue on both sides of the IT, the average

Figure 3. (A) Cumulative BW gain over 1 yr by maximum infection level of a given pasture. Infection level was corrected using Ju et al. (2006) and temperature-humidity index (THI) data for each month was derived from the weather data collected for each study. ADG was calculated using equation 7 with inputs for THI and infection level based on 60 ppb [E] threshold. (B) Cumulative BW gain over a theoretical fall growing season. (C) Cumulative BW gain over a theoretical spring growing season.

depression in ADG for each was 356 and 354 g/d (Parish et al., 2003; Parish et al., 2013), whereas the model predicted average ADG depressions of 262 and 431 g/d. The effect of endophyte-infection level on ADG should be an important consideration for farmers when grazing cattle.

When deciding whether to plan for spring or fall calving (cow/calf) or grazing (stocker) seasons, the effects of endophyte infection level should be considered. One study specifically compared the profitability of fall-calving vs. spring-calving herds and concluded that, when grazing endophyte-infected fescue, it is more cost effective to calve in the fall (Caldwell et al., 2013). The same study went on to note that higher daily gain could be seen in spring-calving herds if the pasture was nontoxic endophyte-infected tall fescue (Caldwell et al., 2013). Multiple studies have shown the increased profitability of calving in the fall, due in part to higher daily gain for calves (Bagley et al., 1987;

McCarter et al., 1991; Gaetner et al., 1992; Henry et al., 2016). The present study also supports the idea that farmers maintaining pastures with endophyte-infected tall fescue should consider switching to a fall calving system as a means to minimize reductions in BW gain.

Limitations

The biggest limitations of this meta-analysis were sample size and data structure. Two weighting strategies were employed to combat this limitation by including more studies with incomplete data reporting. Some of the recorded variables did not have consistent distributions. For example, there were no data for studies beginning in 5 mo of the year, which limited ability to derive effects by change in climate. Another result was an inconsistent distribution of trial durations for each starting month, making it hard to establish a duration

effect. The inconsistency of measurement methods required splitting the data into three groups, severely limiting the size of each of the training sets. Ideally, as more studies are conducted on the effects of novel endophyte-infected tall fescue cultivars, a more consistent dataset utilizing one measurement method will become available.

SUPPLEMENTARY DATA

Supplementary data are available at *Journal of Animal Science* online.

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