

Observations of High Nitrate Concentrations in Forage Bermudagrass during Severe to Exceptional Drought Years

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Abstract

With the prediction of climate change-induced increases in drought frequency and severity in the southeastern USA, it is important to better understand the risks that drought may pose to NO₃ accumulation in bermudagrass [*Cynodon dactylon* (L.) Pers.] forage. This report offers observations of NO₃ concentration in Bermudagrass forage samples submitted to the University of Georgia's Feed and Environmental Water Lab (FEWL) during the extreme to exceptional drought of 2007, the severe drought of 2008, and the four preceding seasons when drought stress was minimal or absent. The probability (*P*) of a sample being at high risk for nitrate toxicosis was the greatest for the extreme to exceptional drought of 2007 (*P* = 0.160), slightly lower in the severe drought year of 2008 (*P* = 0.105), and the lowest for samples from the 2003-2006 growing seasons (*P* = 0.082) when drought stress was minimal or absent.

Keywords

Nitrates, Drought, Bermudagrass

1. Introduction

Global climate change is expected to cause an increase in frequency and severity of drought in the southeastern United States during the next century [1]. One of the risks posed to forage production by an increased frequency

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and severity of drought in this region would be the potential for a toxic build-up of nitrate-nitrogen ($\text{NO}_3\text{-N}$) concentrations in the forage crops common to this region [2]. Ruminants that consume a diet consisting of more than $2500 \text{ mg NO}_3 \text{ kg}^{-1}$ are at risk of developing abnormally high concentrations of nitrite in their bloodstream, leading to the formation of methemoglobinemia. This, consequently, can result in condition commonly referred to as nitrate toxicosis. Nitrate levels higher than $4500 \text{ mg NO}_3 \text{ kg}^{-1}$ pose a risk of chronic nitrate toxicosis and, in some situations, animal death [2].

Bermudagrass [*Cynodon dactylon* (L.) Pers.] is the most widely used warm season perennial grass for pasture and hay production in the southern USA, and it is noted for its drought tolerance and high water use efficiency [3]. Further, bermudagrass is highly efficient at using N and most of the nitrate reductase activity occurs in its roots [4]. Though this may reduce its risk in accumulating toxic concentrations of NO_3 [5], there is sufficient evidence to suggest that toxic NO_3 levels in bermudagrass do frequently occur [6]-[10]. However, it is unclear how frequent one might expect to find bermudagrass forage samples to be high in nitrates, especially during severe to exceptionally severe drought.

Recent weather conditions in Georgia (USA) have granted a unique opportunity to make observations about the propensity for NO_3 accumulation in bermudagrass. The National Drought Mitigation Center's U.S. Drought Monitor classified a large majority of the state of Georgia in their extreme (D3) to exceptional (D4) drought categories during the 2007 growing season and at least a severe (D2) drought during 2008 ([11]; **Figure 1**). These years had been preceded by four years of relatively little prolonged drought stress. In each of these years, a substantial number of forage samples were being submitted for forage quality and nitrate analysis at the University of Georgia's Feed and Environmental Water Lab (FEWL). As a result, a very large and robust database exists that could provide insight into the nitrate concentration characteristics of forage submissions made during drought years and years when drought stress during the growing season was absent or much less pronounced. Thus, the objective of this work was to offer *in actu* observations from the University of Georgia's FEWL on the nitrate concentration of bermudagrass forage samples submitted during the drought years of 2007 and 2008 and the four preceding seasons when drought stress was minimal or absent.

2. Materials and Methods

This study uses observations obtained from the analyses of bermudagrass forage samples submitted to the University of Georgia's FEWL from 1 July 2003 through 31 March 2009. The data were first screened to include only those samples that had been analyzed for NO_3 concentration, which is determined in the FEWL by a colorimetric analysis of the nitration of salicylic acid [12]. Data were further screened to eliminate samples from areas that had not experienced similar levels of drought stress during 2007 and 2008. Pasture condition reports

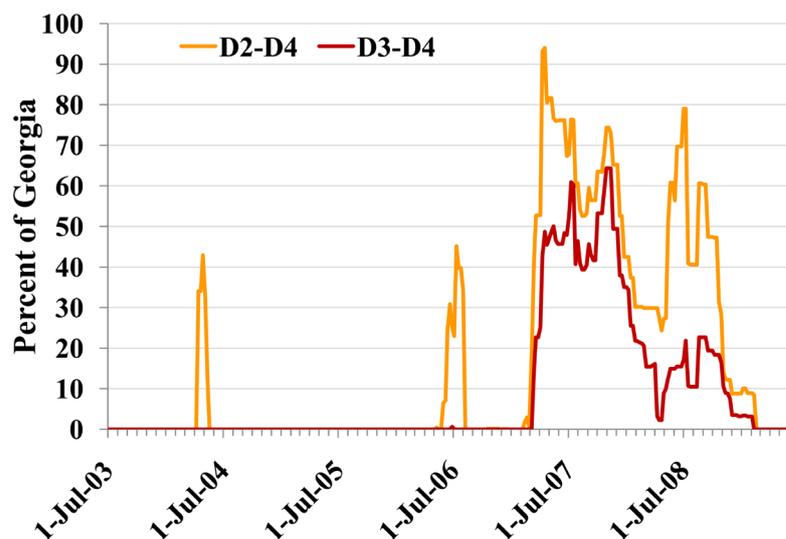


Figure 1. The percent of the state of Georgia (USA) that was classified by the national drought mitigation center's U.S. drought monitor (2009) as being in extreme to exceptional (D3-D4) and severe to exceptional (D2-D4) drought categories between 1 July 2003 and 30 June 2009.

from the USDA—National Agricultural Statistics Service (NASS) indicated that drought conditions were not consistently severe in Georgia’s counties along the Atlantic Coast during 2007 and 2008. Therefore, all observations from the 17 counties in the NASS’s Georgia District-9 were screened from each year used in this analysis.

Next, the data were segregated into growing seasons based on sample submission date, using an assumption that bermudagrass from an individual growing season would be submitted between 1 June of that same year and 31 March of the following year. For example, bermudagrass samples submitted between 1 June 2007 and 31 March 2008 were categorized into the “2007” growing season category. All other observations falling outside of this time frame (*i.e.*, with a sample submission date of 1 April through 31 May) were excluded from the analysis. Samples submitted from the 2003, 2004, 2005, and 2006 seasons were originally subjected to an analysis similar to that described below but were found to not differ ($P > 0.10$) from one another (data not shown). Because of their similarity and indications from U.S. Drought Monitor data that bermudagrass forage produced during this period was largely free from severe drought stress, the observations from the 2003, 2004, 2005, and 2006 growing seasons were combined into one large category (“2003-2006”).

Using the UNIVARIATE procedure in SAS [13], the screened nitrate data were found to exhibit significant skewness and kurtosis (Table 1) and, therefore, violated assumptions of normality. Consequently, the data were subjected to a natural logarithmic transformation. Further, approximately 20% of the observations in the complete database were found to have nitrate levels below the 100 mg NO₃ kg⁻¹ detection limit. These observations of nitrate levels below the detection limit are known as left-censored data and were included in this analysis by employing a survival analysis technique. In this technique, the censored data are kept in the analysis but are treated differently from actual observed values. Specifically, it is assumed that there is a latent variable Y_i^* for the i -th observation which is the actual NO₃ concentration in the natural log scale, while Y_i is the detected natural log transformed NO₃ concentration. The model can be expressed using Equations (1) to (3), and used within the LIFEREG procedure in SAS [13], which is used to fit survival data.

$$Y_i^* = \beta_0 + \beta_1 I\{\text{year} = 2007\} + \beta_2 I\{\text{year} = 2008\} + \epsilon_i, \epsilon_i \sim N(0, \sigma^2); \quad (1)$$

$$Y_i = Y_i^* \text{ if } Y_i^* > \ln(100) \quad (2)$$

$$Y_i = \ln(100) \text{ if } Y_i^* \leq \ln(100) \quad (3)$$

where β_0 , $\beta_0 + \beta_1$, and $\beta_0 + \beta_2$ are the means of the true ln transformed NO₃ concentrations in the null, 2007, and 2008 growing season categories, respectively; $I\{\text{year} = 2007\}$ is an indicator variable which is 1 for growing season 2007 and 0 for other growing seasons and $I\{\text{year} = 2008\}$ is an indicator variable which is 1 for growing season 2008 and 0 for other growing seasons (*i.e.*, for the null growing seasons, the two indicator variables are both zero).

In order to compare the frequency of having moderate or highly risky NO₃ concentrations within the growing season categories, the probabilities of these occurrences during the individual growing seasons were calculated. This was done by first grouping the bermudagrass samples into three categories of toxicity risk based on their NO₃ concentration: Low Risk (*LR*), where nitrate levels were less than 2500 mg NO₃ kg⁻¹; Moderate Risk (*MR*), where nitrate levels were 2500 - 4500 mg NO₃ kg⁻¹; and High Risk (*HR*), where nitrate levels greater than 4500 mg NO₃ kg⁻¹. Because the three levels are ordinal, a cumulative logistic model [14] was used to estimate the log (base e) probability (P) of a bermudagrass forage sample being in a particular risk category in the 2003-2006,

Table 1. Distribution characteristics of the observations used in the analysis of the NO₃ concentrations in bermudagrass forage samples submitted to the UGA-FEWL during the 2003-2006, 2007, and 2008 growing season categories.

Growing season	n	Range	Skewness	Kurtosis	W^\dagger	D^\ddagger
		mg NO ₃ kg ⁻¹				
2003-2006	2533	ND [§] - 18,647	1.969	5.385	-	0.258**
2007	1010	ND - 17,804	2.503	8.507	0.808***	0.181**
2008	869	ND - 17,012	2.981	12.623	0.716***	0.238**

** , *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively. [†] W = the Shapiro-Wilk test statistic, which is appropriate for characterizing datasets when $n \geq 2000$. [‡] D = Kolmogorov-Smirnov statistic. [§]ND = not detectable.

2007, and 2008 growing seasons. Specifically, Equation (4) presents the cumulative logistic model for the log odds that was analyzed using the LOGISTIC procedure in SAS (SAS Institute, 2004).

$$\log odds_j = \alpha_j + \tau_1 I \{ \text{year} = 2007 \} + \tau_2 I \{ \text{year} = 2008 \}, j = 1, 2 \quad (4)$$

where the cumulative logits are defined as Equations ((5) and (6)).

$$\log odds_1 = \text{logit} [P(\geq MR)] = \ln \frac{P(\geq MR)}{1 - P(\geq MR)} = \ln \frac{P(MR) + P(HR)}{1 - P(MR) - P(HR)} \quad (5)$$

$$\log odds_2 = \text{logit} [P(HR)] = \ln \frac{P(HR)}{P(LR) + P(MR)} = \ln \frac{P(HR)}{1 - P(HR)} \quad (6)$$

where intercepts α_1 and α_2 are associated with $\log odds_1$ and $\log odds_2$ (as defined above), respectively, and have the interpretation of log odds in the 2003-2006 growing seasons, τ_1 is the increment to the log odds in the 2007 growing season, and τ_2 is the increment to the log odds in the 2008 growing season; where $I \{ \text{year} = 2007 \}$ is an indicator variable that equals 1 for the 2007 growing season and 0 for other growing seasons and $I \{ \text{year} = 2008 \}$ is an indicator variable that equals 1 for growing season 2008 and 0 for other growing seasons (*i.e.*, for the 2003-2006 growing seasons, the two indicator variables are both zero); and where it is given, as defined in Equation (7), that the probabilities for each risk category in a given year sum to equal 1.

$$P(LR) + P(MR) + P(HR) = 1 \quad (7)$$

For each growing season category (2003-2006, 2007, and 2008), the estimated parameters from Equation (4) were used in Equation (5) to calculate the probability of *MR* or greater [$P(\geq MR)$] concentrations of NO_3 in the submitted forage samples. Similarly, these model parameters were then used in Equation (6) to calculate the probability of *HR* [$P(HR)$] concentrations of NO_3 in the sample submissions for each growing season category. The probability of *MR* [$P(MR)$] NO_3 concentrations was derived by subtracting $P(HR)$ from $P(\geq MR)$. Then the probability of *LR* [$P(LR)$] NO_3 concentrations was derived from Equation (7) using the estimates of $P(HR)$ and $P(MR)$.

3. Results and Discussion

3.1. *In Actu* Observations of Nitrate Concentrations in Bermudagrass

The results of the analysis of maximum likelihood estimates for β_0 , β_1 , and β_2 indicated that all parameters were significantly ($P < 0.01$) greater than zero (Table 2). Since β_0 , $\beta_0 + \beta_1$, and $\beta_0 + \beta_2$ are estimates of the means of the true natural log transformed NO_3 concentrations during the 2003-2006, 2007, and 2008 growing season categories, respectively, these estimates were calculated and transformed back to the original units (Table 3). The mean concentration of NO_3 in samples submitted during the 2003-2006 growing seasons (512 mg $\text{NO}_3 \text{ kg}^{-1}$) was significantly lower ($P < 0.05$) than in samples submitted from the drought-stressed season of 2008 (626 mg $\text{NO}_3 \text{ kg}^{-1}$), and both were significantly lower than those from the extreme to exceptional drought-stressed season in 2007 (1170 mg $\text{NO}_3 \text{ kg}^{-1}$). Though these mean values are considerably lower than those expected to pose a moderate (2500 mg $\text{NO}_3 \text{ kg}^{-1}$) or high (4500 mg $\text{NO}_3 \text{ kg}^{-1}$) risk of nitrate toxicosis [2], each growing season had a substantial number of samples with NO_3 concentrations that would be considered moderate or highly risky (Table 4).

3.2. Estimates of the Probability of Low, Moderate, and High Nitrate Concentrations

To compare the frequency of moderate or highly risky NO_3 concentrations across growing season categories, the probabilities of these occurrences during the individual growing seasons were calculated. The results of the analysis of maximum likelihood estimates for parameters α_1 , α_2 , τ_1 , and τ_2 used in Equation (4) indicated that each of the parameters was significantly different ($P < 0.01$) from zero (Table 5). These parameters were used to estimate the probabilities of *LR*, *MR*, and *HR* concentrations of NO_3 in bermudagrass forage sample submissions during the 2003-2006, 2007, and 2008 growing seasons (Table 6).

In each of the three growing seasons, there existed a high probability ($P > 0.65$) that a bermudagrass forage sample would be classified as *LR*. However, this probability differed between each growing season category,

Table 2. Results of the analysis of maximum likelihood estimates for β_0 , $\beta_0 + \beta_1$, and $\beta_0 + \beta_2$, which are parameters in the models (Equations (1) to (3)) that estimate the means of the true log (base e) transformed NO_3 concentrations during the 2003-2006 (null), 2007, and 2008 growing season categories, respectively.

Parameter	Estimate	95% CI	SE	χ^2	$P > \chi^2$
β_0	6.237	± 0.066	0.034	34,303.4	<0.0001
β_1	0.827	± 0.122	0.062	177.59	<0.0001
β_2	0.196	± 0.130	0.066	8.78	0.0030

Table 3. Means and 95% confidence intervals for the NO_3 concentrations during the 2003-2006, 2007, and 2008 growing season categories.

Growing season	Mean	95% CI	SE
	mg NO_3 kg^{-1}		
2003-2006	512 ^{af}	± 32.6	16.79
2007	1170 ^c	± 200.1	102.09
2008	626 ^b	± 110.4	56.33

[†]Same letters within column are not significantly different at $P < 0.05$.

Table 4. The frequency with which bermudagrass forage samples submitted to the UGA-FEWL were at low, moderate, and high risk for nitrate toxicosis during the 2003-2006, 2007, and 2008 growing seasons.

Growing season	Low risk (< 2500 mg NO_3 kg^{-1})	Moderate risk (2500 - 4500 mg NO_3 kg^{-1})	High risk (> 4500 mg NO_3 kg^{-1})
2003-2006	80.3% (2034/2533)	11.2% (284/2533)	8.5% (215/2533)
2007	65.2% (659/1010)	19.6% (198/1010)	15.1% (153/1010)
2008	75.8% (659/869)	13.3% (116/869)	10.8% (94/869)

Table 5. Results of the analysis of maximum likelihood estimates for α_1 , α_2 , τ_1 , and τ_2 , which are parameters in the models (Equations (4) to (6)) used to estimate the probability of low, moderate, and high risk NO_3 concentrations during the 2003-2006, 2007, and 2008 growing season categories in **Table 6**.

Parameter	Estimate	95% CI	SE	Wald χ^2	$P > \chi^2$
α_1	-1.402	± 0.097	0.0497	793.7724	<0.0001
α_2	-2.411	± 0.120	0.0614	1541.714	<0.0001
τ_1	0.756	± 0.160	0.0817	85.5141	<0.0001
τ_2	0.263	± 0.182	0.0928	8.0623	0.0045

Table 6. The calculated probability (and 95% confidence intervals) of a submitted bermudagrass forage sample being categorized as a low, moderate, and high risk for nitrate toxicosis during the 2003-2006, 2007, and 2008 growing seasons.

Growing season	Low risk (< 2500 mg NO_3 kg^{-1})	Moderate risk (2500 - 4500 mg NO_3 kg^{-1})	High risk (> 4500 mg NO_3 kg^{-1})
2003-2006	0.802 \pm 0.0159	0.115 \pm 0.0063	0.082 \pm 0.0086
2007	0.656 \pm 0.0602	0.184 \pm 0.0213	0.160 \pm 0.0342
2008	0.757 \pm 0.0549	0.138 \pm 0.0231	0.105 \pm 0.0251

with the highest probability occurring in the more “typical” growing seasons of 2003-2006 (0.802 \pm 0.0159), followed by the severe drought season of 2008 (0.757 \pm 0.0549), and with the lowest probability occurring in the extreme to exceptional drought of 2007 (0.656 \pm 0.0602). The most concerning issue revealed in this compari-

son was that samples submitted from the 2007 growing season were 1.95 times (0.160/0.082) more likely to be in the high risk category than in the “normal” growing seasons of 2003-2006. Though this also tended ($P < 0.10$) to occur in 2008, it was not as likely as in 2007.

3.3. Limitations, Additional Key Findings, and the Need for Additional Research

It is important to note that our results should not be interpreted as establishing the expected mean NO_3 concentration of samples from similar seasons or number of observations in *LR*, *MR*, and *HR* categories during drought. Our analysis was performed on producer-submitted bermudagrass forage samples. It is likely that producers were more apt to identify and submit forage samples from bermudagrass lots that may have been at an increased risk of nitrate toxicity during the droughts of 2007 and 2008. This may have increased the observed mean and altered the distribution of NO_3 concentrations in the samples from these seasons. Even so, this analysis has shown that high risk samples do frequently occur and that this may even occur in samples from growing seasons that were not severely drought stressed (**Table 1**, **Table 4**, and **Table 6**).

Moreover, these observations indicate that NO_3 may accumulate in bermudagrass to a much higher level than previously thought. Of the published work that reports NO_3 concentration in forage bermudagrass [6]-[9], the highest reported level was $8350 \text{ mg NO}_3 \text{ kg}^{-1}$ [8]. In the 2003-2006 growing seasons when relatively little prolonged drought stress was present, we observed that 1.86% (47/2533) of the samples had NO_3 concentrations greater than $8350 \text{ mg NO}_3 \text{ kg}^{-1}$. In the extreme to exceptional drought of 2007 and the severe drought season of 2008, we observed that 3.47% (35/1010) and 2.30% (20/869) of the bermudagrass samples submitted from those respective growing seasons exceeded this previously reported high.

The degree to which drought stress influences NO_3 concentrations in forage bermudagrass will need to be assessed in well-designed field trials. For example, this additional research should include an assessment of the influence of N fertilizer application strategies (e.g., splitting the applied N equally across multiple applications, the use of “enhanced-efficiency” and slow/controlled-released fertilizers, etc.) so that an assessment can be made of the degree to which N management interacts with the growing season conditions to influence the development of toxic NO_3 concentrations in bermudagrass.

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