Genetics of fescue toxicity in Angus cattle: Identification of differentially expressed genes in tolerant and susceptible Angus cows^.

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Summary

Fescue toxicosis (FT) is the multifaceted syndrome that caused the major loss of revenue in beef industry. The objective of this study was to identify differentially expressed (DE) genes due to difference tolerance level to FT in Angus cows. Forty pregnant purebred Angus cows were selected based on their growth at two locations (high and moderate levels of toxic fescue), and classified as either high tolerant (HT) or low tolerant (LT) to FT with 20 cows in each group balanced by location. Blood samples were collected on weeks 1,5,9, and 13 for RNA sequencing. Normalized gene counts were analysed with a negative binomial model. Genotype-by-environment interaction was evident in the study. There were more DE genes ($P < 0.01$) between HT and LT animals at one location (high toxic levels; 550) than the other (moderate; 83). At greater toxic level, DE genes were enriched for relevant functions such as cardiac, protein metabolism, stress response, and other metabolic-related functions, supporting that FT has implications on vasoconstriction, immune response, and digestive capacity in cattle. Altogether, the study of blood transcriptomic for FT in beef cattle may be used to identify candidate genes to improve response to FT.
Keywords: RNA-seq, host genomics, fescue toxicosis

Introduction

Tall fescue (*Festuca arundinacea*) is the most commonly cultivated forage in the United States to feed the beef cattle. Approximately, 90% of total tall fescue pastures in the US are infected with the fungal endophyte *Neotyphodium coenophialum* that causes a disease in the animals called fescue toxicosis (FT; Browning, 2003). Cattle suffering from FT may have reduced pregnancy rates and calving rates, rough hair coats, heat stress, poor growth, reduced hair shedding, reduced appetite, insufficient flow of blood to the extremities, and more. With these symptoms, FT causes loss of about $1 billion in the U.S. beef industry (Browning, 2003).

Although genetic variation could be utilized to manage this disease, little is known about the host genetic control of fescue toxicity. Browning (2000) reported no differences in cortisol and prolactin concentration, respiration rate, rectal temperature, skin temperature between Hereford and Red Brahman steers injected with ergotamine. Senepol steers were less susceptible to heat stress than Hereford steers and had greater ADG when compared to Hereford steers when consuming the endophyte infected diet (Browning, 2004). Gray et al. (2011) reported a moderate heritability (0.35) for the first month of hair shedding in Angus cattle under toxic fescue. In addition, this trait had a negative genetic correlation (-0.58) with weaning weight. However, the genetic mechanisms that could explain this variation are still unknown. The objective of this work was to identify differentially expressed (DE) genes due

to different tolerance level to FT in Angus cows.

Material and methods

Data collection

Purebred, multiparous, pregnant Angus cows (n = 149) were taken from two beef herds from North Carolina: Butner Beef Cattle Field (BBCF; Butner, NC, USA) and Upper Piedmont Research Station (UPRS; Piedmont, NC, USA). Half of the tall fescue pasture in BBCF was infected with 95% endophyte and other half was infected with 65% endophyte. At UPRS 95% of the tall fescue pasture was infected with endophyte. Weekly data (from April 26 to July 19, 2016) were collected for weight. Blood samples (10 ml) of each cow were collected from all cows via jugular venepuncture into Tempus Blood RNA Tubes on weeks 1,5,9 and 13. Animals were selected based on the growth during the trial, as the slope of regression analysis of body weight on weeks (average weekly gain; AWG). A total of 40 animals (20 from each location) were selected based on AWG during the first 7 weeks, as detailed by Galliou et al. (2017). In each location 10 cows were classified into either high tolerant (HT) and low tolerant (LT) genetic groups.

RNA extraction, sequencing, and bioinformatics

Total RNA from the 40 selected cows was extracted by Tempus™ RNA isolation kit Sequencing was performed in an Illumina NextSeq 500, generating 150 bp paired-end reads. Sequence reads for each sample were mapped to *Bos taurus* UMD3.1.88 reference genome using Bowtie2 (Langmead et al., 2010). A total of 20,000 genes were mapped to the reference genome. After quality control, 15,360 genes were used for the analyses.

Statistical analysis

Gene counts were normalized (TMM; Robinson and Oshlack, 2010) and then analysed with a negative binomial model including the fixed effects of genetic group, location, time, RNA integrity number (covariate), flow cell, and interactions of genetic group by location and genetic group by time and a random effect of animals, using a first order autoregressive covariance structure, using SAS 9.4. List of significant genes ($P < 0.01$) were used to perform gene enrichment analysis in PANTHER (Thomas et al., 2003) for biological and molecular functions.

Results and discussions

There were several significant DE genes (4,617) between the two locations, suggesting that the differences in toxicity between locations (greater % of infected-fescue at UPRS) can largely impact the blood transcriptomic profile of pregnant cows. Because of this major location impact, we expected to see differences in DE genes between genetic groups at each location, which could explain the large number of DE genes for the interaction between genetic group-by-location.

When genetic group was compared within each of the locations, there were 550 and 83 DE genes between HT and LT animals at UPRS and BBCF, respectively, supporting the presence of genetic-by-environmental effect, with a much greater number of DE genes at

when toxic levels were greater (i.e. at UPRS). With that, we decided to focus on DE genes between genetic groups within each location. There were no biological and molecular functions associated with the DE genes at BBCF ($P > 0.05$). However, at UPRS (greater toxicity), there were 10 and 7 biological and molecular functions, respectively, overpresented ($P < 0.05$) for the DE genes between HT and LT animals (Table 1). The overpresented gene functions included: fertilization, protein production and different metabolic functions. Molecular functions like response to stress, protein metabolic process, cellular protein modification, growth factor activity protein binding, calmodulin binding could be related to the vascular functions and loss of appetite, which are the symptoms of FT. These results agree with Tanaree et al. (2013), who reported a large number of genes associated to protein production, cellular metabolic pathway, and cardiac development in steers fed ergovaline.

Table 1. Enrichment analysis for DE[1] genes between genetic groups at UPRS[2]

Functions	No. of genes	FE[3]	*P*-value
Biological function			
Protein metabolic process	67	1.35	0.008
Glycolysis	5	5.89	0.002
Transmembrane receptor protein	5	3.39	0.017
Signal transduction	63	1.34	0.014
Cell communication	69	1.31	0.013
MAPK cascade	16	2.3	0.002
Intracellular signal transduction	35	1.46	0.017
I-kappaB kinase/NF-kappaB cascade	5	3.01	0.027
Response to stress	33	1.66	0.004
Cellular protein modification	41	1.64	0.001
Molecular Function			
Growth factor activity	5	3.87	0.020
Protein binding	77	1.23	0.033
Calmodulin binding	12	2.36	0.003
Phospoprotein phosphatase activity	10	2.26	0.015
Phospatase activity	12	1.86	0.031
Ubiquitin – protein ligase activity	10	2.22	0.017
Ligase activity	16	1.67	0.033

[1]DE genes = Differentially expressed; UPRS[2] = Upper Piedmont Research Station; FE[3] = Fold-enrichment

The list of top 3 genes overexpressed for HT and LT groups at UPRS is shown in Table 2. Of these genes, S100A3 is found in hair cuticle which binds with calcium and zinc and helps in formation of keratin protein and could also be associated to hair coat shedding. MYL2 and LOX genes have cardiac functions, which might play role in the force of heart contraction and regulation of force of cardiac muscle contraction suggesting the response to vasoconstriction. G-protein coupled receptor protein is associated with congestive heart failure which is stimulated during period of stress. This could be associated with the irregularities in blood circulation during fescue toxicity in cows. The other genes have general biological functions, but their high expression and associations put them as candidate genes for genetic tolerance to FT.

Table 2. Top overexpressed genes for High-(HT) and Low-Tolerant (LT) groups at UPRS[1].

Gene symbol	Log_2FC [95% CI]	Gene name	*P*-value
HT			
U6	1.44 [1.24, 2.56]	U6 spliceosome RNA	0.0002
S100A3	1.44 [1.12, 2.80]	S100 calcium binding protein A3	0.0070
OLIG1	1.45 [1.16, 2.28]	Oligodendrocyte transcription factor 1	0.0010
LT			
GPRCD5	-2.76 [-4.84, -1.48]	G protein coupled receptor class 5	0.0007
MYL2	-1.81 [-2.98, -1.26]	Myosin light chain 2	0.0018
LOX	-1.60 [-2.90, -1.10]	Lysyl oxidase	0.0056

[1]UPRS = Upper piedmont research station; Log_2FC = log_2 fold change. Positive and negative values represent over expression in the HT and LT groups, respectively; 95% CI = 95% confidence interval

Conclusions

Genotype-by-environmental interactions were evident in this study. The analysis of significant genes showed that most of these genes were responsible for growth, cardiac function, protein metabolism and stress response. The results in the study widens the knowledge about the genetic control of fescue toxicosis in Angus cows.

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