

The Development of a Genetic Marker for Resistance to Fescue Toxicosis in Beef Cattle

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Abstract

Fescue Toxicosis is currently one of the most costly diseases that beef producers in the eastern United States are facing. The syndrome is caused by a fungal endophyte that infects tall fescue, the predominant forage in Tennessee. Fescue Toxicosis in cattle can cause fescue foot, rough, thick hair coat in the summer, decreased feed intake, and decreased serum prolactin levels, which drastically affect reproductive ability (Roberts and Andrae, 2004). Studies have indicated that binding of ergopeptides, the active compounds produced by endophyte, to dopamine 2 receptors (DRD2) may be responsible for many of the deleterious effects of fescue toxicosis. The overall objective of this project was to develop a genetic marker that provides resistance to fescue toxicosis in beef cattle. An intronic A→G Single Nucleotide Polymorphism (SNP) was found in a region of the DRD2 gene, creating the genotypes AA, GG, and AG. To test the informativeness of this SNP for resistance to fescue toxicosis, hair follicles from Angus and Angus-cross steers (n = 32) and bulls (n = 72) that had grazed on endophyte-infected tall fescue were collected. Genomic DNA was isolated and the DRD2 SNP was genotyped through polymerase chain reaction (PCR) followed by Restriction Fragment Length Polymorphism (RFLP) analysis. Phenotypic information on steers included serum prolactin concentrations and body weight measured monthly over April, May, and June of 2006 and 2007. Hair coat scores were recorded for each steer in June. For bulls, hair coat score and reproductive parameters were measured and recorded as they arrived at a central bull test location in August 2007. The reproductive parameters included scrotal circumference, sperm motility and morphology. With AA and AG genotypes in both steers and bulls ($P \leq 0.05$). Steers with the genotype AA had the highest level of serum prolactin, while steers with the genotype GG had the lowest ($P < 0.05$). No relationship was observed between genotype and reproductive parameters in bulls or between genotype and body weight in steers. These data support the usefulness of the DRD2 SNP as a marker for some (but not all) aspects of fescue toxicosis.

Introduction

Fescue Toxicosis is an exceptionally costly disease that affects the beef cattle industry in the eastern United States, resulting in estimated losses to the beef industry of 600 million dollars annually due to lowered conception rates and depressed body weight gains (Patterson et al., 1995; Roberts and Andrae, 2004). Fescue Toxicosis is caused by the ingestion of endophyte infected tall fescue, which is the predominant forage in Tennessee. Fescue Toxicosis causes a number of problems in beef cattle such as fescue foot and a rough, thick hair coat in the summer. The syndrome also causes decreased feed intake, which affects the body weight gains. Fescue Toxicosis also causes a decreased level of serum prolactin in beef cattle, which drastically affects the reproductive ability of the animal (Roberts and Andrae, 2004).

Serum prolactin levels in beef cattle are directly proportional to their reproductive abilities. Reduced levels of serum prolactin have been consistently noted as an indicator of Fescue Toxicosis. Prolactin levels are negatively regulated by dopamine through the DRD2

receptor. Other studies have indicated that the binding of ergopeptides (the active compound produced by the endophyte) to the DRD2 receptors may be responsible for many of the deleterious effects of Fescue Toxicosis.

Variations may be seen in the manifestation of Fescue Toxicosis within a given herd of cattle, leading to the hypothesis that genetic variation may exist which alters an animal's physiological response to the endophyte exposure. Due to the link between prolactin levels as an indicator of Fescue Toxicosis, which is regulated by DRD2, the DRD2 gene is an excellent candidate for containing the genetic information that could possibly code for the resistance to Fescue Toxicosis. Recently, our lab has discovered a sequence variation in the DRD2 gene sequence which may serve as a marker for susceptibility to Fescue Toxicosis. The sequence variation was an intronic A→G single nucleotide polymorphism (SNP) that was found in the region of the DRD2 gene, creating the genotypes AA, AG and GG. In this study, the informativeness of this genetic marker was tested for several indicators of Fescue Toxicosis in beef steers and bulls exhibiting varying degrees of Fescue Toxicosis.

Materials and Methods

Steers grazed on endophyte-infected tall fescue pastures at either the Blount (2006) or Highland Rim (2007) farms of the University of Tennessee Experiment station from the time they were weaned (January) until they were shipped to feedlots (July). Steers were weighed monthly during April, May and June. Also, in June a single blood sample was taken for the analysis of circulating serum prolactin concentrations. Each animal was also scored on their hair coat using a common scoring scale (1=short and smoother; 5= rough, long and shaggy). Tail hairs from which genomic DNA was later isolated were also collected at this time.

Seventy two bulls enrolled in the 2007 University of Tennessee Bull Test located at the Spring Hill Research and Education Center was used. The bulls were assumed to have been grazing on endophyte-infected tall fescue. Hair coat scores (ranging from 1 to 5) were recorded for each animal. Tail hairs were also collected for later isolation of genomic DNA. Breeding soundness exams, which included scrotal circumference, sperm motility and morphology, were performed by a University of Tennessee Extension Veterinarian.

Genomic DNA was isolated from the tail hair follicles of each animal using Quickextract (Epicentre, Cambridge, UK). A genomic amplification was then performed for the isolated DNA samples using the GenomiPhi DNA amplification kit (GE Healthcare, Piscataway, NJ). The GenomiPhi kit uses the bacteriophage Phi29 DNA polymerase to exponentially amplify linear DNA templates using a strand displacement reaction and resulted in the amplification of the entire genome of the animal. An ethanol precipitation was then performed, which further concentrated and purified the DNA sample. After the ethanol precipitation the DNA then provided an archivable stock. A polymerase chain reaction (PCR) was then performed to amplify a portion of the gene of interest (DRD2). Genotypes of the samples were determined by

restriction fragment length polymorphism (RFLP), which allows for the differences in a homologous DNA sequence to be determined by the presence of different lengths of DNA fragments following a sequence-specific digestion with the Tfi I enzyme (New England Biolabs, Ipswich, MA). The enzyme cuts the DNA sequence at the specific polymorphism if it contains an A; if the sequence contains a G it would not be cut. A gel was then run to allow the DNA fragments to separate so that the genotype of the animals could be determined. The genotypes were then compared with the steers and bull's collected data and genotype-phenotype associations were tested.

Results

The DNA sequence of the DRD2 gene with the single nucleotide polymorphism is shown in Figure 1. The yellow highlighted sequences are the primers that were used in the PCR to cut for the gene of interest. The pink highlighted region is the SNP contained in the DRD2 gene.

Figure 1. Sequence description of the DRD2 polymorphism.

TATAGCCCCATTCTGCTTCTTGAAATCCCATGACTCTTAGATTTTAAACCACATGCTTTGAGTCTTCCTGTGTCCTGTC
 CAGTAATGGTCTCCAAAAGGCACGCTGGCATTATGTGCAAGGCATTATGTGAGGCATTATGAGCCCCAGAGGCTCCACA
 TGGCGGCTGTGCCTTCTCTGAGATGCTCCTGACTGCCTCACACAGGACCCGGTCACGATGGGCTAGCCTCTTATTTTTTTC
 TACCTTTTCTGCTCCAGGTGGCTCAGGCCTGGCCTGAGTTTGGCCAGTCCTTGGGGCTGAAGCTTCATTATCAGGGGTGA
 TGGAGGCCAGCAGCCTCCACCCTGGGTGCACACACAGACAGGCACAGACATGCAGTCTGCCAGGCTGTCTGGTTGG
 CTCCAATTTAATAAAGTTGAAAATCACCAGGCATCCTCCTGAAACATTATCCCCATTCTCACCCAACACCATTGTGCTCCA
 AGCCATTTTTCTCTCTGACCTTACCAGGAACAATCCAGGAGACCGGA[G/A]TCACCCTGACCCAGGAGACTCAGCCTGGC
 TTCAGTCTAAGGTGGTGAACAGGCTCTTGGTGGGCCACCACCATGTCCACGCCAGGGTTCAGCTCCCTCAGGGGCCAGC
 TGAGCCCCAGGAGACAGAGGCAGGACGCACCTGTGTGTTGAGTCCGAAGAGCATCGGGCAGGAGATGGTGAAGGACA
 GGACCCAGACGATGGCGATCATGACCGTGACCCGGCGCTTGGAGCTG**TAACGTGTGTGTAGAGCATGGGC**

Figure 2 is a representative RFLP analysis with Tfi I.

Figure 2.

AA AA AG AG GG GG

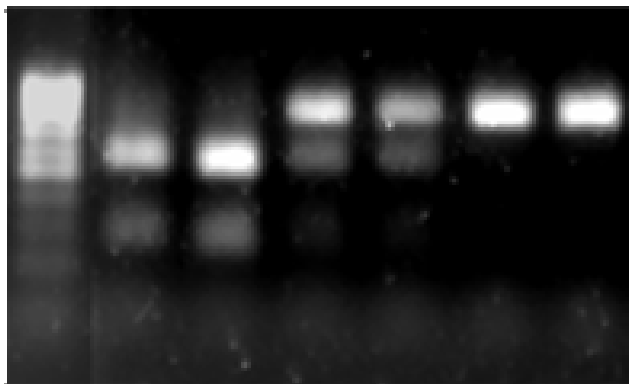
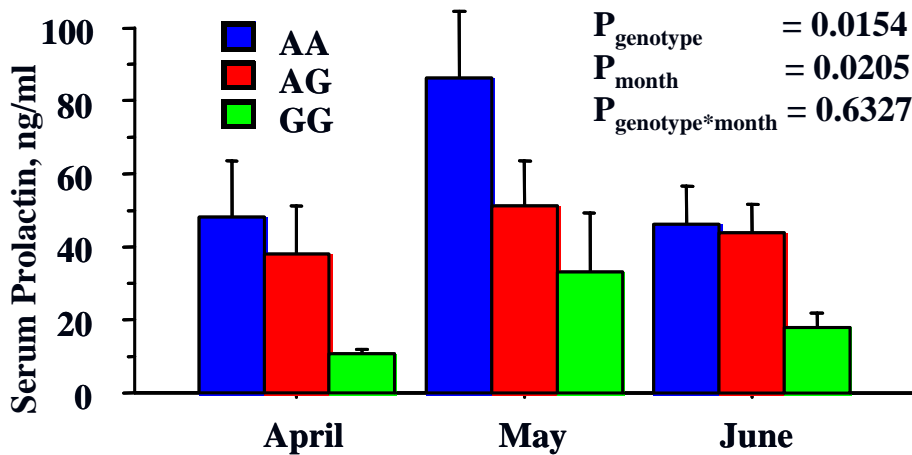


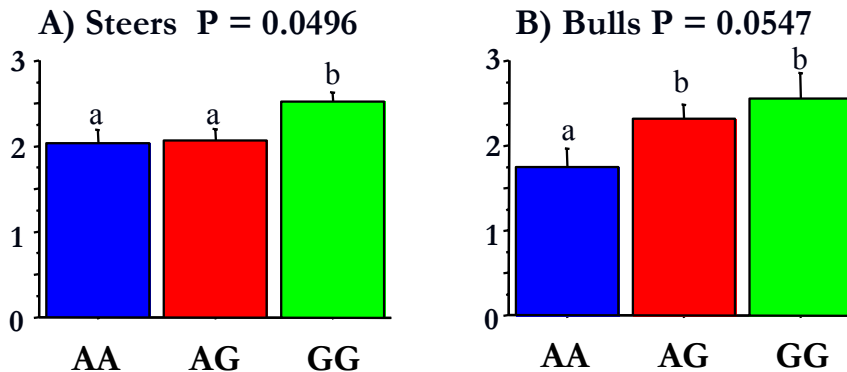
Figure 3 illustrates the relationship between genotype and serum prolactin levels. There appears to be a direct relationship between the levels of serum prolactin and genotype at the DRD2 gene. The “G” allele was noted as the disadvantageous one, as the animals with the “A” allele tend to have higher levels of serum prolactin ($P = 0.0154$). Analysis of variance with repeated measures (Time: April, May and June) was performed with the genotypes and the year as main effects. There was no effect noted of the year so therefore, it was dropped from the model to simplify visualization of results.

Figure 3. Effect of DRD2 genotype on Serum Prolactin Concentrations in steers.



In steers ($P = 0.0496$) and bulls ($P = 0.0547$) there appears to be a direct relationship between hair coat scores and genotypes at the DRD2 gene, with the “G” allele again being disadvantageous. Animals with the “A” allele tend to have lower hair coat scores and therefore, appear to be more resistant to Fescue Toxicosis. Figure 4 illustrates the relationship between the hair coat score and the genotype. Analysis of variance was also performed with genotype and year as main effects and no effect was noted, therefore it was dropped from the model to simplify visualization of results. For bulls and steers genotype was the sole effect on hair coat score.

Figure 4. Effects of DRD2 genotype on hair coat scores in steers (A) and bulls (B). Columns within a graph with different letters differ $P > 0.05$.



The data suggest that the genotype in the DRD2 gene has a significant effect on an animal's ability to be resistant to Fescue Toxicosis. No relationship however, was observed between genotype and reproductive parameters in the bulls (data not shown).

Discussion

The results of this study indicate that the DRD2 gene contains genetic information that affects the animal's resistance to fescue toxicosis. Animals who's DNA contains an "A" allele at the SNP in the DRD2 gene appears to be more resistant to Fescue Toxicosis therefore, the "G" allele is disadvantageous. Further research with additional bulls, steers, cows and heifers must be done to determine the effectiveness of this marker in identifying animals that are genetically resistant to the effects of Fescue Toxicosis.

Conclusion

The purpose of this research project was to determine if there was genetic information in the DRD2 gene that would mark for higher resistance to Fescue Toxicosis in beef cattle. This study indicates that the SNP contained in the DRD2 gene codes for higher resistance to some aspects of Fescue Toxicosis in beef cattle. Further research must be done to fully evaluate the effectiveness of this marker. Increased resistance to the disease will have a great economic impact on the beef cattle industry in the Fescue Belt and particularly in Tennessee. The development of an effective genetic marker for resistance to the syndrome would allow for producers to selectively breed for hardier animals, increasing efficiency and therefore profitability.

References

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- Roberts C, Andrae J. 2004. Tall fescue toxicosis and management. *Crop Management* (online) doi:10.1094/CM-2004-0427-01-MG