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Microarray evaluation of bovine hepatic gene response to fescue toxicosis

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RESEARCH PAPER

Abstract

'Fescue toxicosis' is a disease in livestock caused by ingestion of ergot alkaloids produced by the fungal endophyte *Neotyphodium coenophialum* in tall fescue; it is estimated to cost 1 billion USD in damages per year to the beef industry alone. Clinical signs include decreased reproductive fitness, necrosis of extremities, and reduced average daily gain and milk production. Little is known about the cellular mechanisms that mediate these toxic sequelae. We evaluated the effects of ergovaline-based fescue toxicosis on gene expression via oligonucleotide microarray. Liver biopsies were obtained from steers (n=4) pre- and post-exposure (0 and 29 days) to feed containing 579 ng/g ergovaline. Analyses were performed using both ANOVA with false discovery rate correction and Storey's optimal discovery procedure. Overall, down-regulation of gene expression was observed; heart contraction and cardiac development, apoptosis, cell cycle control, and RNA processing genes represented the bulk of differentially expressed transcripts. 2 CYPs (CYP2E1 and CYP4F6) were amongst the significantly upregulated results. Thus, exposure of cattle to toxic levels of ergovaline caused widespread changes in hepatic gene expression, which can both help explain macroscopic clinical signs observed in ruminant animals, and reinforce previous findings in monogastric models.

Keywords: endophyte, gene expression, ruminants, tall fescue, liver

1. Introduction

In ruminants, 'fescue toxicosis' is induced via consumption of ergot alkaloids produced by an endophytic fungus (*Neotyphodium coenophialum*) in tall fescue (*Festuca arundinacea*). The most common effect is vasoconstriction, which can lead to ischemia and necrosis of the extremities in cold weather (fescue foot), and a reduced ability to thermoregulate during hot weather, resulting in decreased weight gain since less time is spent grazing as the animal seeks shade or water to cool itself (summer syndrome) (Bacon, 1995). Reproductive abnormalities have been correlated with consumption of endophyte-infected grasses as well, including reduced birth rate and milk production (Bacon, 1995; Strickland *et al.*, 2009). Ergovaline is the ergot alkaloid most often associated with causing fescue toxicosis, as it is the ergot alkaloid found in highest abundance in tall fescue (Lyons, 1990).

To date, no survey of gene response to endophyte toxicity has been performed in cattle, though similar studies have been performed in rat and mouse models (Bhusari *et al.*, 2006; Settivari *et al.*, 2006, 2009). These studies demonstrated that genes involved with energy production, growth, development and detoxification were differentially expressed in hepatic tissue from rodents exposed to ergovaline. We hypothesised a similar response would occur in liver tissue from cattle, as well as a response from genes associated with vasoconstriction. Thus, the goal of this study was to determine if bovine transcriptional response to fescue toxicosis was similar to previously observed changes in monogastric model organisms.

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2. Materials and methods

Feeding trial and sample collection

A full description of the feeding trial and experimental procedures associated with it can be found in Merrill *et al.* (2007). Briefly, four yearling (200±6 kg) Angus×Hereford steers were fed chopped, high-alkaloid (579 ng/g ergovaline) tall fescue straw over 29 days, an effective dosage of 11.52 μg/kg body weight per day. Liver biopsies were taken by fine needle biopsy using a sterile, 14 gauge Allegiance Tru-Cut biopsy needle (Allegiance, McGaw Park, IL, USA) on day 0 (pre-exposure) and day 29 (post-exposure). The biopsy area was shaved, washed with disinfectant, iodine and alcohol after which a small incision was made and the biopsy needle inserted for liver sampling. Tissue was preserved immediately in RNAlater solution (Qiagen, Valencia, CA, USA), then stored at -20 °C until RNA extraction.

RNA extraction and probe preparation

RNA was extracted and purified with the RNeasy extraction kit (Qiagen). Quantitation and purity determination were performed on an ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA); A_{260}/A_{280} ratio was at least 2.0 and graph shape was smooth and even for all samples. Extracted whole RNA was amplified via the TargetAmp 1-round RNA amplification kit (Epicentre, Madison, WI, USA) using ~500 ng of each of the 8 samples, with 3 separate amplifications created for each sample. Integrity of the sample was confirmed by gel electrophoresis.

Quantified RNA was normalised to a uniform concentration, then an equal volume of each sample was subjected to reverse transcription and Cy3/Cy5 labelling (GE Healthcare, Piscataway, CA, USA) using an Atlas PowerScript fluorescent labelling kit (Clontech, Mountain View, CA, USA). To control for dye variability, a dye swap was performed on 1 of 3 whole RNA extract amplifications.

Hybridisation

Three technical replicates of each probe pair were created for each of the 4 biological replicates (i.e. 3 replicates for day 0 and day 29 for each individual steer). Probes created from pre- and post-exposure whole RNA extracts with opposing dye types were combined and purified using BD Chroma Spin (Clontech) columns. Probes concentrated to 6 μl via vacuum centrifugation and reconstituted in deionised water were diluted into 35 μl SlideHyb hybridisation buffer #3 (Ambion, Austin, TX, USA), incubated for 5 min at 70 °C, and applied to a prepared bovine microarray slide (National Bovine Functional Genomics Consortium (NBFGC) EST microarray; Suchyta *et al.*, 2003). Slides were placed in a hybridisation chamber and maintained at 55 °C for 15 h, then subjected to 5 post-hybridisation washes (0.5% sodium dodecyl sulphate (SDS), two washes of 0.1× saline-sodium citrate buffer (SSC), 0.01% SDS and 0.1× SSC). A final rinse in 18 MΩ water was performed and slides were dried on a C1303-T slide spinner (Labnet International, Woodbridge, NJ, USA). Slides were immediately scanned using a GenePix Professional 4200A slide scanner (Molecular Devices, Sunnyvale, CA, USA) at 10 μm resolution. Features were extracted with GenePix Pro 6.0 software (Molecular Devices Software, Downington, PA, USA).

Data analysis

Median intensity data were extracted and normalised via a print-tip lowess regression in the maanova R package (Wu *et al.*, 2003) to account for within-slide variation and spatial effects, then normalised for between-slide variation via median centring. Spots that were uniformly of low intensity were filtered. Bad spots, as flagged by GenePix, were identified and substituted via K-nearest-neighbour imputation using the Bioconductor Impute package (k=10). Upregulation and downregulation values were determined using a weighted average, giving flagged bad spots (now imputed) a weight of 0.1, as opposed to a full weight of 1.0 for good spots.

The normalised, collapsed data were then converted via *ad hoc* R scripts for extraction of differential gene expression (EDGE) (Leek *et al.*, 2006; Storey *et al.*, 2007) and significance analysis of microarrays (SAM) (Tusher *et al.*, 2001). For EDGE, which lacks a built-in means of handling technical replicates, the 3 technical replicate results for each of the 4 experimental samples were collapsed into a single result via median calculation. False discovery rate (FDR) analysis was performed with 1000 permutations.

Spots where the conditions $P<0.05$ and $q<0.15$ were met for both analysis packages were compared with the NBFGC's database of BLAST results for array sequences (GeneLink), and a list identifying these results was generated (Supplementary Table S1). Additional annotation was generated using BLAT alignment (Kent, 2002) of NBFGC array sequences and the Ensembl library of human cDNA sequences (Hubbard *et al.*, 2009) via translated sequence. Due to time constraints, no comprehensive study was done on the sensitivity of the NBFGC array with our modified hybridisation protocol to levels of change; probe entries were left as-is with no explicit fold-level filtering performed.

Subsequent lookup of Gene Ontology (GO; Gene Ontology Consortium file dated 7/1/2009; Ashburn *et al.*, 2000) and Kyoto Encyclopedia of Genes and Genomes (KEGG; file downloaded 7/1/2009) pathway annotation was performed via a series of Perl scripts. In this case, lists were determined by comparing all differentially expressed genes with $q<0.15$, then clustering by both GO annotation codes and KEGG pathways via R scripts. Accuracy of

homologue identification was performed via a Perl script that compared GO and KEGG descriptions and tallied overlaps in description codes. An R script was utilised to identify overexpressed GO and KEGG annotations by comparing the number of hits for each term in significant results against a permutation-based (n=10,000) probability generated from the array annotation database, minus control spots. Additional verification was performed via comparison against a hypergeometric distribution against the full set of annotated probes as a further check for overexpression.

Quantitative RT-PCR validation

Validation of randomly selected spots (filtered for high statistical significance in all differential expression analyses (P<0.05 and q<0.15), extremity of changes (fold change >1.5), and existence of notation information) was performed using quantitative reverse transcription polymerase chain reaction (qRT-PCR). Some exceptions were made on selection: NBFGC_BE752999, NBFGC_BE721581 and NBFGC_AW353561 represented transcripts of interest and so were added to the list directly. β-actin was chosen as an endogenous control due to consistent expression and intensity across arrays. The reaction used was the one-step RNA-to-Ct kit for TaqMan chemistry (Applied Biosystems, Foster City, CA, USA); an ABI Prism 7500 thermocycler (Applied Biosystems) was used to collect data. Control efficacy was determined by running duplex reactions with other probes at multiple levels of dilution and confirming a consistent ratio. Probe and primer sequences were generated using IDT SciTools (http://www.idtdna.com/Scitools/Applications/RealTimePCR/), with manual screening of results for product length, annealing temperatures, and presence of hairpins (Supplementary Table S2). Resulting sequences were compared against the nr non-redundant BLAST database (Pruitt *et al.*, 2007) to ensure specificity. Products of each primer pair produced single bands on an agarose gel, supporting single product formation.

Each probe was measured in triplicate for each original, unamplified RNA extract in a duplex reaction with the β-actin control to normalise experimental readings. The resulting Ct values were used for pre- and post-exposure state comparison via a paired 2-sided t-test with assumption of unequal variance as performed in R 2.7.1.

3. Results and discussion

Ergot alkaloids induce heat sensitivity via vasoconstrictive effects and reproductive problems that can range from spontaneous abortion to reduced fecundity (Porter and Thompson, 1992). In addition, more minor effects can include changes to the immune system, fat necrosis, and alteration in insulin regulation (Bacon, 1995; Jang, 1987). Hormonally, ergot alkaloids act as D2 dopamine receptor agonists, which inhibit the release of prolactin, a hormone important for lactogenesis (Strickland, 2009). Thus, suppressed serum prolactin levels are often used to diagnose cases of fescue toxicosis. Little is known about the molecular-level mechanisms that mediate these toxic sequelae; a better understanding of these mechanisms could aid in the development of more effective preventative or therapeutic measures. Previous genomic studies in other organisms are useful, but may not capture a fully accurate picture due to the use of monogastrics (rat and mouse models; Settivari *et al.*, 2006, 2009 and Bhusari *et al.*, 2006, respectively), which have fundamentally different digestive systems than ruminants. Therefore, this study aimed to better understand the aetiology of fescue toxicosis in cattle exposed to toxic levels of ergovaline, and determine if the results concurred with what has been observed in rodent models.

Gene expression analysis

Due to the low sample number, it was intended that the intersection of two methods for determining differential gene expression (SAM and EDGE) would give the most accurate data. For both methodologies, downregulated transcripts (12,791 of 18,816 spots (68%)) were predominant, but the largest number of overlaps occurred in probes for upregulated transcripts (Figure 1). This may be due to the relative paucity of upregulated results, coupled to the fact that such results are shared. Correlation between the 2 lists reinforced the conclusion of differential expression for each overlapping probe.

Overlapping probes with EDGE-calculated significance values and BLAT-extrapolated annotations can be found in

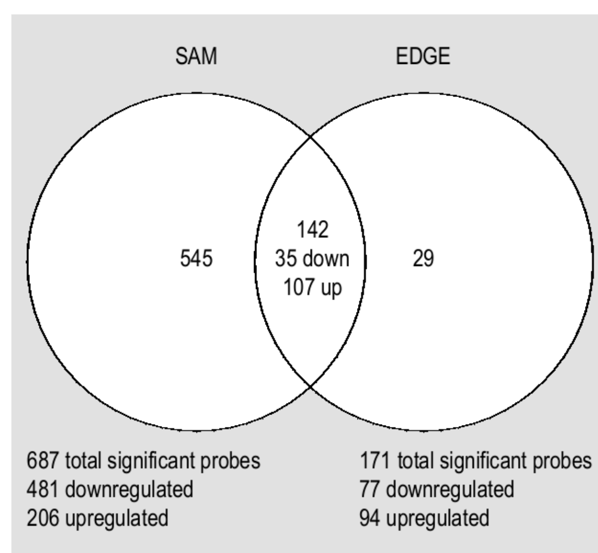

Figure 1. Venn diagram comparing results of significance analysis of microarrays (SAM) to extraction of differential gene expression (EDGE) methods of data analysis.

Table 1, sorted by q-value. An abbreviated list of the 20 most significant results is given in Table 1. Results were filtered to remove all but 1 of the GAPDH control spots, which dominated downregulated transcripts. This eliminated it as an endogenous control, in favour of β-actin. This choice is reinforced by Robinson *et al.* (2007), who found GAPDH and β-actin to be the best candidates for bovine endogenous controls. The BLAT search against the bovine genome yielded 9,336 annotations, of which 5,133 were unique and not in the original GeneLink database. Accuracy was extrapolated by comparing GO description codes of the new hits against existing GO codes in GeneLink. Within 4,528 annotated sequences, 4,016 had a majority (>50%) of their ontology codes overlap. The 512 hits that did not match tended to have a single GO code assigned to the NBFGC GeneLink annotation, the Ensembl annotation, or both, though the gene descriptions were frequently similar. This suggested that annotation was not detailed enough to gain an accurate picture of gene function. As annotation improves, these 512 transcripts could contribute additional information as to the molecular events occurring in response to ergovaline ingestion.

Major overrepresented categories affected in cattle livers exposed to ergovaline-containing forage are listed in Table 2. The main functional GO categories identified were heart contraction, intracellular transport, apoptosis, and RNA transcription control. Significant KEGG pathways found were inositol metabolism and oxidative phosphorylation, confirming previous results (Settivari *et al.,* 2006, 2009). Results of validation via qRT-PCR are outlined in Figure 2A, and were used to confirm microarray results. They generally followed the pattern of microarray results, but suffered from high sample-to-sample variance, indicating a large degree of variation existed between animals. Examining results on an array-by-array basis, however, revealed fairly close

Table 1. Twenty most differentially expressed genes in hepatic tissue from steers fed 579 ng/g ergovaline over 29 days listed by FDR-corrected significance.

Probe[1]	GENE_ID[2]	Description	Ensembl[3]	*P*-value[4]	*q*-value[4]	log (fold change)
NBFGC_BF775357	NA[5]	NA	NA	<0.001	0.003	4.15
NBFGC_BE899799	618232	similar to PC2-glutamine-rich-associated protein	ENSBTAP00000029374	<0.001	0.003	3.68
NBFGC_BF600471	506860	similar to 6-pyruvoyl tetrahydrobiopterin synthase precursor	NA	<0.001	0.003	3.36
NBFGC_BF652151	507901	similar to G-2 and S-phase expressed 1	NA	<0.001	0.003	2.22
NBFGC_AW668942	767882	hypothetical protein LOC767882	ENSBTAP00000005656	<0.001	0.003	2.15
NBFGC_BE752002	789168	similar to ligand-gated ion channel	ENSBTAP00000051245	<0.001	0.003	2.15
NBFGC_BE810106	NA	NA	ENSBTAP00000017949	<0.001	0.003	2.07
NBFGC_BF889822	NA	NA	NA	<0.001	0.003	2.06
NBFGC_BF655238	NA	NA	NA	<0.001	0.003	0.97
NBFGC_BF193682	NA	NA	NA	<0.001	0.003	0.79
NBFGC_AW668885	526516	hook homolog 2	ENSBTAP00000043388	<0.001	0.003	0.4
NBFGC_BE723767	407121	nebulin	ENSBTAP00000053811	<0.001	0.003	-2.57
NBFGC_BE899799	618232	similar to PC2-glutamine-rich-associated protein	ENSBTAP00000029374	<0.001	0.003	-4.85
NBFGC_BF602720	NA	NA	NA	<0.001	0.004	4.02
NBFGC_BG690396	326578	SNW domain containing 1	ENSBTAP00000035416	<0.001	0.004	2.86
NBFGC_AW668803	513924	similar to CD2 binding protein 1 long form; CD2BP1L	ENSBTAP00000028179	<0.001	0.004	2.81
NBFGC_BF606553	535372	β-sarcoglycan (Beta-SG)	ENSBTAP00000052639	<0.001	0.004	2.68
NBFGC_AW668882	618184	KDELR1 KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 1	ENSBTAP00000013035	<0.001	0.004	2.65
NBFGC_AW655532	NA	NA	NA	<0.001	0.004	2.63
NBFGC_BE754415	540396	HIVEP2 human immunodeficiency virus type I enhancer binding protein 2	ENSBTAP00000015933	<0.001	0.004	2.55

[1] National Bovine Functional Genomics Center probe accession number.
[2] Entrez accession number.
[3] Ensembl protein accession number.
[4] *P*- and *q*-values listed from EDGE calculation.
[5] NA = not available.

Table 2. Patterns of gene expression identified using Gene Ontology and Kyoto Encyclopedia of Genes and Genomes databases in hepatic tissue from steers fed 579 ng/g ergovaline over 29 days.

Gene Ontology term	Count[1]	Percentage[2]	P-value[3]
GO:0007507~heart development	7	1.63%	0.002
GO:0006412~translation	24	5.58%	0.012
GO:0006396~RNA processing	15	3.49%	0.012
GO:0060047~heart contraction	4	0.93%	0.015
GO:0043068~positive regulation of programmed cell death	7	1.63%	0.017
GO:0043065~positive regulation of apoptosis	7	1.63%	0.017
GO:0042398~amino acid derivative biosynthetic process	4	0.93%	0.019
GO:0016071~mRNA metabolic process	10	2.33%	0.024
GO:0002026~regulation of the force of heart contraction	3	0.70%	0.032
GO:0016072~rRNA metabolic process	5	1.16%	0.035
GO:0006364~rRNA processing	5	1.16%	0.035
GO:0043009~chordate embryonic development	5	1.16%	0.035
GO:0019395~fatty acid oxidation	3	0.70%	0.042
GO:0048193~Golgi vesicle transport	5	1.16%	0.043
GO:0012502~induction of programmed cell death	5	1.16%	0.043
GO:0006357~regulation of transcription from RNA polymerase II promoter	6	1.40%	0.046
Kyoto Encyclopedia of Genes and Genomes term			
bta00562:Inositol phosphate metabolism	6	1.40%	0.002
bta00190:Oxidative phosphorylation	13	3.02%	0.004
bta04070:Phosphatidylinositol signaling system	7	1.63%	0.005
bta03050:Proteasome	4	0.93%	0.07

[1] Number of transcripts in larger superset falling into a given category.
[2] Percentage of transcripts out of significant results.
[3] Significance of results as determined via a hypergeometric distribution comparison against the full set of transcripts on the array.

matches between arrays from the same biological sample, and frequent overlaps resulting in P>0.05 (for example, glutathione S-transferase mu 1 (GSTM1), Figure 2B).

Vasoconstriction is the root cause of 2 sequelae contributing to ergot-based cattle losses. Ergot alkaloids act as α-adrenergic and serotenergic agonists that stimulate smooth-muscle contraction, resulting in an inability to regulate blood flow to extremities, which is important in thermoregulation. This can manifest in a number of ways, as previously described (Porter, 1995; Strickland, 2009). In this study, there were an unusually large number of genes associated with cardiac development and action (supplementary data available upon request). Heart contraction and regulation of the force of heart contraction as categories were significant (Table 2) suggesting a response to vasoconstriction. Interestingly, one of our downregulated probes (NBFGC_BF193711) matched sequence most closely for a heat shock response transcription factor, indicating ergovaline possibly plays a more direct role in heat-induced cardiac stress via reducing effectiveness of heat shock protein (HSP) production. This interpretation might be supported by significant downregulation found in HSP of the rat study by Settivari *et al.* (2006), though in that case, the most significantly change was crystalline alpha B. In addition, the expression of genes regulating inositol phosphate metabolism and the phosphatidylinositol signalling system (Table 2) could be related to vasoconstriction as well, as inositol and its phosphates play a role in modulation of serotonin activity and themselves modulate cardiac activity (Van Heugten *et al.*, 1996). Dyer (1993) indicated serotonin receptors were activated by ergovaline, which may contribute to the vascular effects seen in fescue toxicosis.

There was a strong expectation that cytochrome P450s (CYPs) would be differentially expressed. Settivari *et al.* (2006, 2009) and Bhusari *et al.* (2006) established this in mouse and rat models, and biochemical assays performed by Moubarak and Rosenkrans (2000), Moubarak *et al.* (2003), and Settivari *et al.* (2008) also indicated that this was part of the mammalian xenobiotic response to ergot alkaloids. Unexpectedly, only 3 transcripts were determined via ontology data to be related to detoxification. Of these, there were 2 CYP and 1 glutathione pathway-related transcripts. One of the CYPs was a putative protein similar to CYP4F6 (NBFGC_BE721581). In rats, the main observed biochemical activity of CYP4F6 was in the metabolism of leukotriene

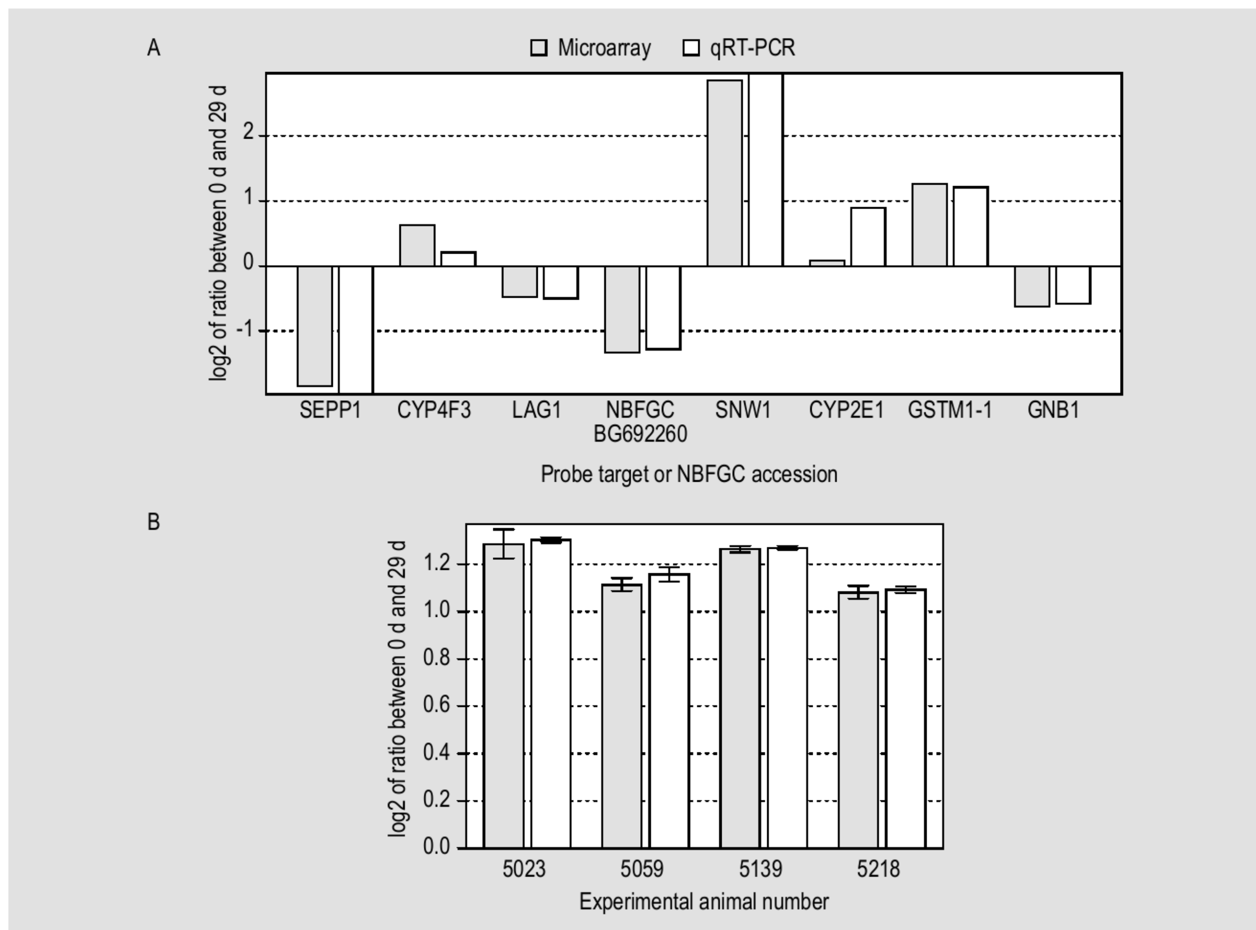

Figure 2. (A) Mean microarray expression vs. mean quantitative real-time PCR expression of 8 selected genes. (B) Microarray vs. quantitative real-time PCR results for glutathione S-transferase mu 1 (NGFGC_BE752999 (GSTM1)) measured separately for each animal. Error bars represent mean of 4 replicates ± standard error.

B4, a lipid mediator with a role in inflammatory response (Bylund, 2003). The other CYP transcript was CYP2E1 (NBFGC_AW353561), a hepatic hydroxylase (Johansson, 1988). In rats and humans, this enzyme was upregulated after exposure to a variety of xenobiotic compounds, including ethanol (Caro and Cederbaum, 2004). The resulting oxidative stress is thought to contribute to liver damage (Kessova and Cederbaum, 2003). The upregulation of these CYPs support previous findings of CYP upregulation in fescue toxicosis (Settivari *et al.*, 2006), but appear to run in the face of later work, which found the inverse (Settivari *et al.*, 2009). Differences in animal health, dosage, and environmental conditions (e.g. animals in the second study were under heat stress) may account for this variability, but further investigation into the role these enzymes play in development of fescue toxicosis is warranted.

The categories of cell cycle control and RNA processing were consistently significant in both the list of differential genes expressed and the GO pathways identified. Of the transcripts related to RNA processing, SNW1 (NBFGC_BG690396) was one of the most significant results obtained in this study. This gene transcribes part of the spliceosome and plays a role in transcription regulation and RNA splicing (Prathapam *et al.*, 2001). Its human homolog, SKIP, is considered of interest in both oncogenesis (Prathapam *et al.*, 2002) and HIV-1 propagation (Brès *et al.*, 2009). This was upregulated, which was unexpected, given that it normally acts as a promoter activator and the majority of transcripts were downregulated. It is possible that activity of this particular protein was masked by other downregulation signals, or that what it promotes is not involved with downregulated categories. Four other results appeared in this category as well (NBFGC_BF655006, NBFGC_BE809185, NBFGC_BE751726, NBFGC_AW668709), however, annotation on these entries was limited.

The wider net cast in performing the GO and KEGG analyses (Table 2) yielded a number of results, suggesting enrichment of apoptosis-related and fatty acid oxidation transcripts. These are notable for matching prior findings (Settivari *et al.*, 2006, 2009). Excessive apoptosis results in tissue necrosis, which is a contributing factor in fescue foot. In addition, oxidation of fatty acids is the process by

which fatty acids are broken down to provide energy in the cell (Mathews *et al.*, 2000).

Summary and future directions

This was the first study to examine changes in gene expression in cattle exposed to ergovaline. As sample number was restricted due to the high cost of running a large-animal study, statistical power was addressed using a combination of statistical programmes. The experiment also showed high animal-to-animal variability in array results, which was confirmed with qRT-PCR validation (Figures 2A and 2B). This may be due to the borderline toxic levels of ergovaline used (576 ng/g; 400-700 ng/g is the threshold of toxicity; Tor-Agbidye *et al.,* 2001). In addition, individual variability in xenobiotic metabolism could account for this, as exemplified by the presence of poor to ultra-rapid metabolizers present in a given population. This results in dramatically different induction/inhibition of xenobiotic metabolizing enzymes, causing varying levels of metabolic conversion for a particular toxin (Ogilvie *et al.,* 2008). For example, when examining expression on an array-by-array basis for the enzyme GSTM1, a close relationship existed between array and qRT-PCR results from the same biological sample (Figure 2B); standard error bars always overlapped, indicating a Student's *t*-test of $P>0.05$. Consequently, there may be additional differentially expressed genes of interest in response to ergovaline exposure, which could be identified in future studies using higher levels of ergovaline in the feed and an increased number of experimental animals. Additionally, the examination of cardiac and vascular tissues and post-translational confirmation of differentially expressed proteins in general could provide information for the refinement of the aetiology of fescue toxicosis.

5. Conclusions

Lowess normalisation and use of 2 data analysis methods for determining differential expression provided a glimpse into the complex changes in hepatic gene expression that occur at the onset of fescue toxicosis. Namely, heart contraction and cardiac development, apoptosis, cell cycle control, and RNA processing genes were significantly altered in response to ergovaline-containing forage consumed by cattle over a 29 day period. These data serve to support prior results found in rats and mice, reinforcing the relevance of those models should they be needed, or more accessible, for experiments. Future studies can refine these findings through the use of better annotated arrays, information derived from completion of the bovine genome, and existence of technologies such as RNAseq, which promise to provide a far more detailed picture of transcriptional changes.

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Supplementary material

Supplementary material may be found online at http://dx.doi.org/10.3920/WMJ2012.1482.

Table S1. Overlapping probes determined to be significant between extraction of differential gene expression (EDGE) and significance analysis of microarrays (SAM) evaluations of differential expression in hepatic tissue from steers fed 579 μg/kg ergovaline for 29 d. Statistical values are those as calculated in EDGE.

Table S2. Probes and primers utilized for quantitative RT-PCR validation.

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