ALFALFA AND MRLS: WHAT WE KNOW AFTER ONE YEAR OF TESTING

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INTRODUCTION

The onset of mare reproductive loss syndrome in April 2001 in Kentucky lead to much speculation about its cause. Some of these initial 'suspects' included the ergot alkaloids of tall fescue, potential cyanide content of white clover, wide Calcium/Potassium ratios in pasture, fungal mycotoxins and the presence of certain poisonous plants. Certain consultants also indicated that no one should purchase or feed any Kentucky hay in 2001. However, these statements were not based on any hard information, just one person's opinion. But great damage was done to the confidence in Kentucky grown hay.

Why was Kentucky hay or pasture suspected to be related to MRLS in 2001?

The main reason for this accusation was the widespread and sudden nature of MRLS in late April of 2001, which pointed to something in the environment or diet of almost all farms in Central Kentucky. Too often (at least for me) pasture and forage get blamed for livestock problems when no other cause is forthcoming. But it was apparent that something in the environment of many or most central Kentucky horse farms was causing severe abortions.

In 2001, an early leading theory for the cause of MRLS was toxicity due to fungal mycotoxins. Mycotoxins are compounds produced by certain types of fungi (such as Fusarium and Aspergillus) when they grow and then are subjected to environmental stress. In 2001, it was theorized that these fungi (which are commonly present in the soil and pasture in Kentucky) were stressed by the late frosts which lead to the production of mycotoxins. The symptoms caused by these mycotoxins were consistent with several of those seen with cases of MRLS, including abortions.

Mycotoxins are difficult to quantify because they are present in very small concentrations (parts per billion). There are also many mycotoxins that potentially might be present, Finally, mycotoxins are water soluble and would be washed off by rain. To complicate the 2001 situation, rain occurred in Kentucky on the Sunday after the Kentucky Derby but before many samples could be taken from problem fields. Therefore there was no way to get a good idea of the mycotoxin profile of pastures during the time when MRLS was occurring.

Fortunately, a lot of hay was made prior to the derby, and this could be analyzed for mycotoxins. With the help of the Kentucky Department of Agriculture and several county agents, samples were pulled from lots of hay made prior to the Kentucky Derby to be analyzed for potential mycotoxin contamination. These samples would help address questions about Kentucky hay and also should get an estimate of potential mycotoxins present on forage prior to the rain after the derby. These samples included both alfalfa and orchardgrass hays. ***No mycotoxins were detected in any sample.*** However, because the cause of MRLS was still not understood, concerns about Kentucky hay persisted.

2002 Monitoring of Pastures and Hayfields

A pasture monitoring program was designed during the fall and winter of 2001-02 to provide data on several pasture and soil characteristics during the spring of 2002. This information would be correlated with information about the heath and reproductive status of mares on these pastures. Pastures that contained mares both early and late in gestation (both groups aborted during 2001) were monitored on several farms across central Kentucky. The 2002 MRLS monitoring program began Feb. 21 and ended June 28. During that time data and samples from 12 horse farms (Thoroughbred and Standardbred) and 1 hay production farm (alfalfa and timothy for horses) were collected and analyzed.

The following were the primary characteristics or measurements taken on monitor farms:

1. Yeast and mold counts in soil, and characterization of the types of fungi present.
   This test was expected to be a measure of the potential of a pasture to produce mycotoxins given the necessary environmental conditions.

2. Cyanide content in white clover:
   While not generally known, white clover can contain cyanide containing sugars that can release cyanide when the cell contents are mixed, such as occurs after a very hard frost. The level of cyano-sugars is determined mainly by the variety of white clover - in other words it is genetically determined. Environmental conditions will raise or lower the content of these compounds in white clover. They are at a seasonal high in the good growth conditions of spring.

3. Nitrate content of the general pasture:
   Nitrates can cause abortions, so this was measured in composite samples that represented the entire pasture.

4. Ergot alkaloid content of tall fescue:
   Endophyte infected tall fescue produces certain alkaloids known to be toxic to horses, and lead to prolonged gestation, foaling difficulty, lack of

milk and even death of the mare and foal. These symptoms are not similar to those of MRLS, but many thought that tall fescue might be involved. So the percent of tall fescue was noted in pastures and samples were taken for alkaloid measurement.

5. Ratio potassium to calcium:
   Another theory of MRLS suspected wide K/Ca ratios in the pasture of pregnant mares. It was felt that heavy fertilization especially with nitrogen and/or potassium on problem farms led to abnormally high K/Ca ratios.

6. Mycotoxins:
   Pasture and hay samples were again measured for mycotoxins in 2002.

GENERAL RESULTS AND CORRELATION OF PASTURE MEASUREMENTS TO MRLS

Summary of Sentinel Farm Activities

- An average of 9 visits per farm, 13 farm visits every 2 weeks.
- An average of 175 samples per farm which does not include blood and urine samples.
- Six of the monitored farms collected blood (378) and urine (205) samples for future analysis.
- Over 3000 samples were collected, including blood and urine and emergency farm samples.
- A total of 83 fields were sampled from the 13 sentinel farms, averaging 6.4 fields/farm.
- Six of the 12 sentinel horse farms experienced losses:
  - 29 early fetal losses and 9 late fetal losses, between 4/25 and 6/13/02.
- UK personnel spent over 1700 person-hours of actual time on monitoring activities.

Summary of Emergency Farm Activities

Other farms were visited when veterinarians or farm managers found MRLS type symptoms and referred them to UK. These included:

Nine farm visits, of which 6 had MRLS cases totaling 27 early fetal losses and 3 late fetal losses. Other farms included 1 control (farm with BCT and no losses) and 2 with concerns because of losses in 2001.

22 fields sampled resulting in 204 additional samples.

Correlations of Pasture Data to MRLS

Data collected by the monitoring program eliminated most pasture characteristics as primary causes of MRLS, such as presence of poisonous plants, cyanide content of white clover, fungal mycotoxins, soil microbiology, weather and mineral content of forages. The monitoring program also strongly confirmed the link between MRLS and the presence of ETC, while finding a possible involvement of endophyte infected tall fescue with late fetal losses on certain farms.

Statistical analysis of monitoring data found significant correlations between cases of MRLS and the presence of black cherry trees in proximity to pasture. There was also an indication that tall fescue alkaloids might be related to some late term abortions (not MRLS) on farms without clear exposure to eastern tent caterpillar or black cherry trees.

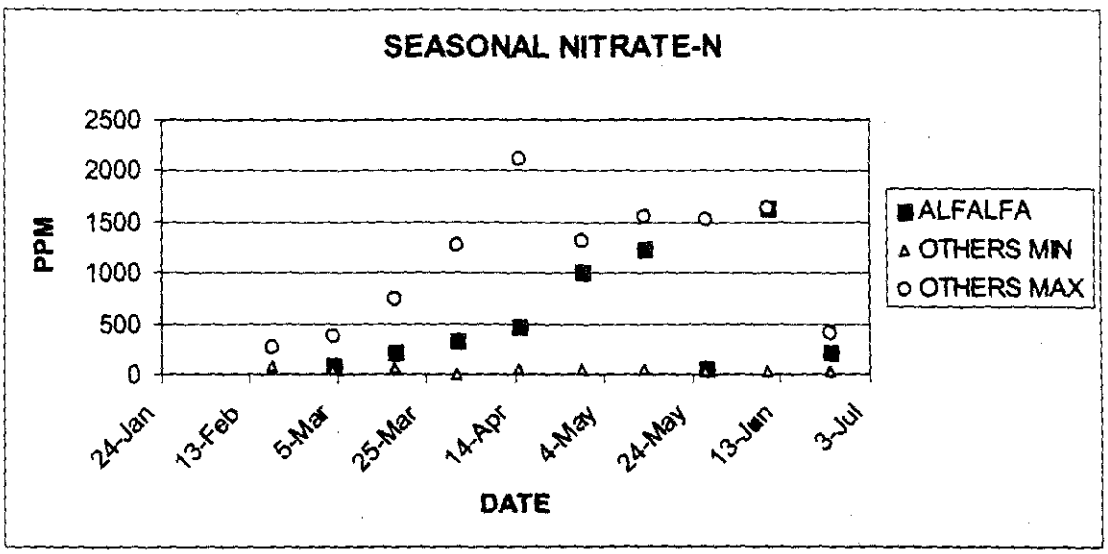
Summary of Pasture Parameters Compared to Alfalfa for Hay Production

Due to research by Dr. Bruce Webb and Karen McDowell from the University of Kentucky, the eastern tent caterpillar was proven to be able to reproduce the symptoms of MRLS in pregnant mare's in the early spring of 2002. This work was repeated and verified by UK scientists in cooperation with private veterinary practitioners in the summer and fall of 2002. This collective work is the strongest evidence that Kentucky grown alfalfa hay is not part of the primary cause of MRLS. However, the monitoring program did gather much data in 2002 that would allow the comparison of alfalfa hay (fresh samples taken direct from the field) to horse pastures across central Kentucky.

The following pages contain the alfalfa values compared to the minimum and maximum across all samples during the indicated time period for most of the parameters measured by the 2002 UK Pasture Monitoring Program. A few notes follow to aid in interpretation of each pasture characteristic or graph.

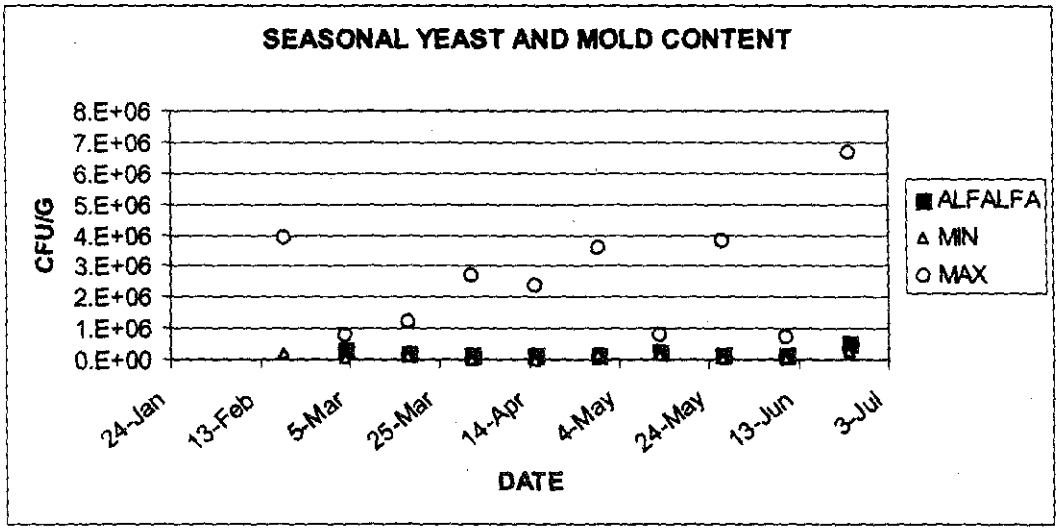
Nitrate from Composite Pasture

The results presented are the concentration of nitrate - nitrogen (NO3-N) in the overall composite pasture sample, expressed in parts per million (ppm). Nitrate can cause toxicity (including asphyxiation and abortion) by being converted to nitrite by the microorganisms in the gastrointestinal tract of livestock. In the horse, this would occur in the cecum. Because most nitrate is absorbed before the hind gut, the horse is much less sensitive to nitrate content of pasture than ruminants. The levels reported for cattle (ruminants) considered to be generally safe are anything less than 1200 ppm. Horses would have a much greater tolerance than cattle. Nitrate levels in all pastures were generally low.



Yeast and Mold Comparison

Yeast and mold counts were determined in the soil from monitored pastures. These are reported in colony forming units per gram (CFU/g). The numeric format for CFU/g used on the graphs is a shortened version of scientific notation. For example, 6.00E+05 is equivalent to 6.00 X 105 or 600,000 CFU/g. All counts were between 200,000 and 2,000,000 CFU/g. High levels of yeasts and especially molds in soil might be a good predictor of a field's potential to produce fungal mycotoxins if environmental conditions were suitable. Alfalfa in 2002 had very low populations of yeasts and molds compared to horse pasture samples.

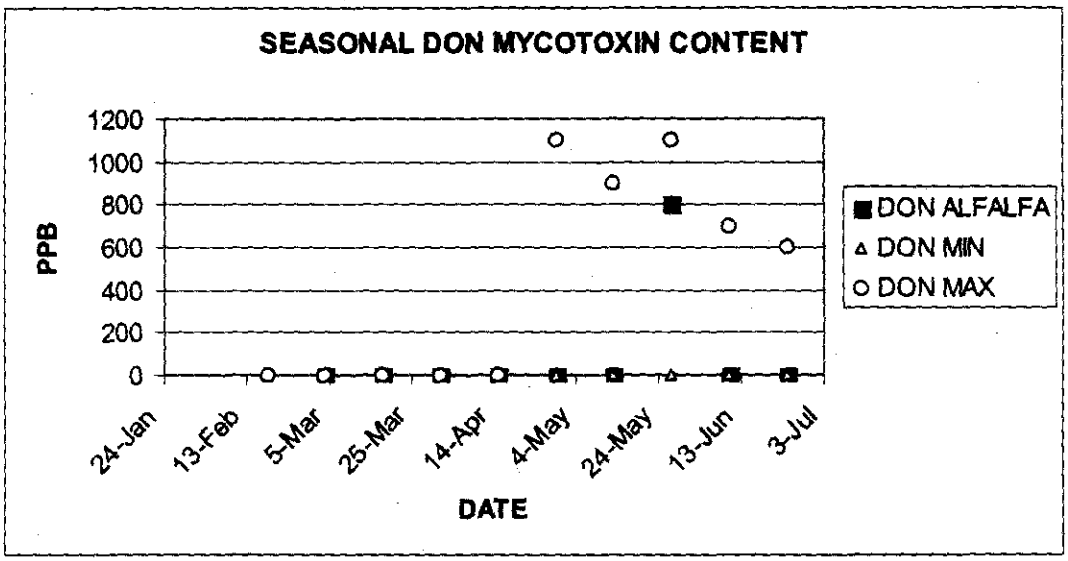


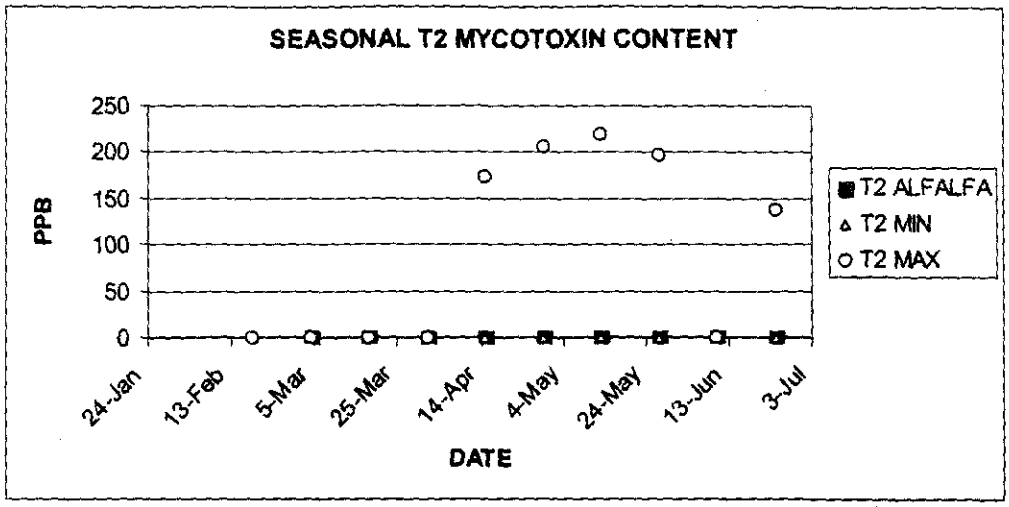
Mycotoxins

Samples from each field were analyzed for the presence and level of several fungal mycotoxins (listed below with their detectable limit) and all data is reported in ppb (parts per billion). Most fields had no detectable mycotoxins. The three principal compounds that did appear in a few pastures were DON (deoxynivalenol), T-2, and Zearalenone (abbreviated ZEA on graph). A 'zero' value on the graph means that concentrations were below the detectable limit. Zearalenone below 500 ppb in the absence of measurable levels of other mycotoxins has not been documented to cause any ill effects.

Alfalfa from the monitoring farm was almost completely free of mycotoxins during 2002. There was only one sample that contained a positive mycotoxin assay (DON, May 24 sample).

Mycotoxins screened for and the detection limit of the assay used: Aflatoxin B1, B2, G1, G2 (Detection limit 1ppb), Fusarenon-X (Detection Limit 1.0 ppm), 3-acetyl DON (DL 0.2 ppm), 15-acetyl DON (DL 0.2 ppm), DON ((DL 0.2 ppm), Nivalenol (DL 1 ppm), Neosolaniol (DL 1 ppm) , diacetoxyscirpenol (DAS) (DL 0.750 ppm), HT-2 Toxin (DL 0.2 ppm), T-2 Toxin (DL 0.2 ppm), Zearalenone (DL 0.2 ppm), Fumonisin B1, B2, B3 (DL 0.1ppm), Ochratoxin A (DL 1ppb).

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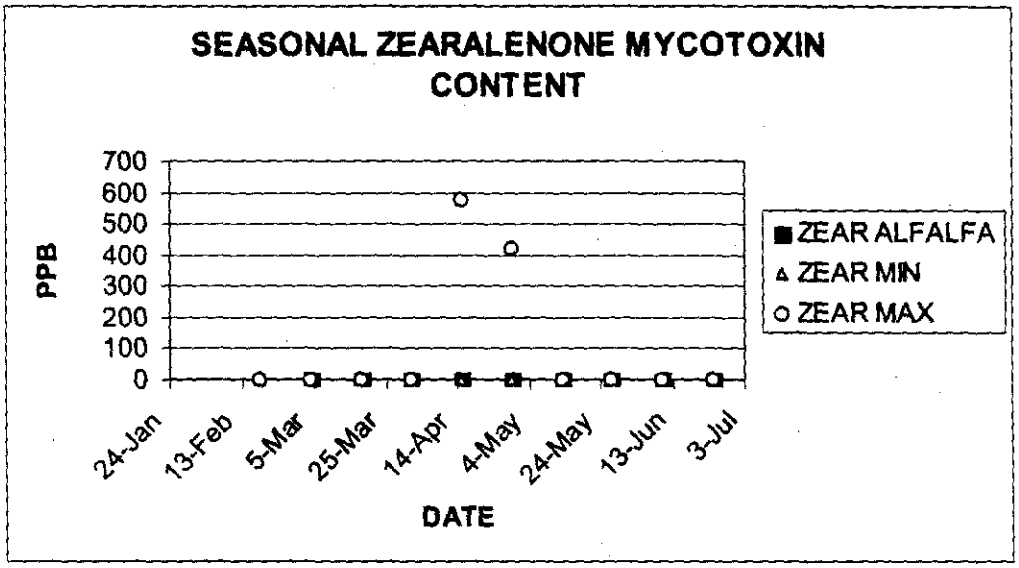
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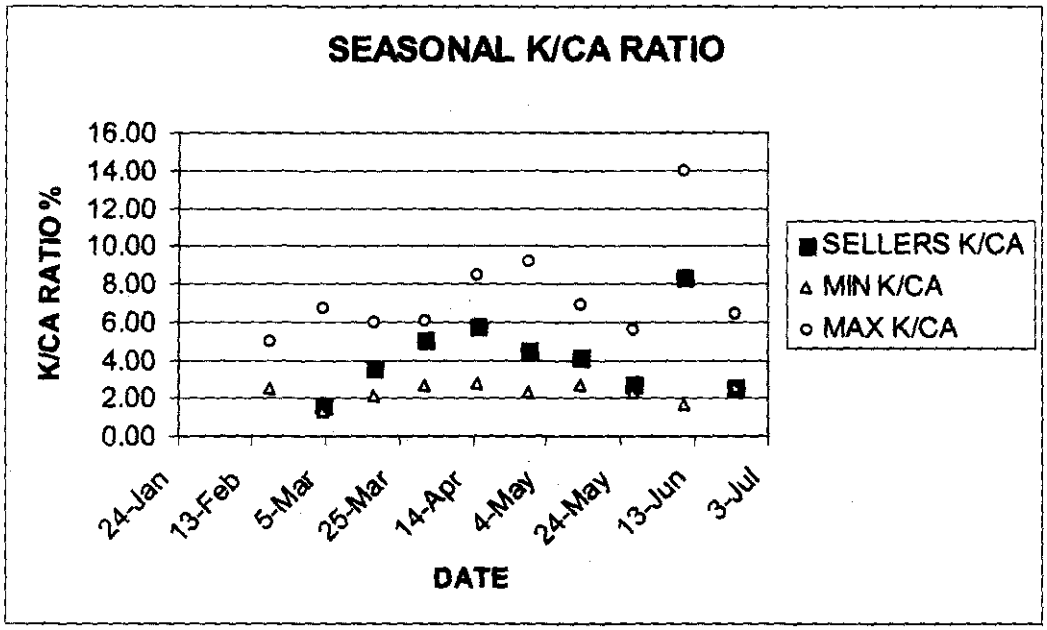
Potassium/Calcium Ratio (K/Ca)

One early theory for the cause of MRLS was that pastures in 2001 had excessively high ratios of potassium to calcium. Values for K/Ca greater than 5:1 were suggested to lead to mineral imbalances in the pregnant mare. K/Ca values for most sampling dates were less than 5 for all pastures and never exceeded 7. For perspective, the average K/Ca ration for May 2001 across several farms was 6.76 to 1. However, the K/Ca for 1996 for the same farms in May was 7.58 to 1.



Summary

Much was learned about the seasonal composition of central Kentucky horse pastures compared to a comparison alfalfa field in the spring and summer of 2002. The alfalfa monitored in 2002 had low and nutritionally safe nitrate levels that were below the maximum reported from general pastures during all sampling periods except one. Yeast and mold counts were low compared to the maximum noted on general composite pasture samples. Mycotoxin values were generally zero across the whole sampling period. The absence of antiquality factors in alfalfa and the ground breaking research by UK scientists Drs. Bruce Webb and Karen McDowell that directly implicated the eastern tent caterpillar as the primary cause of MRLS indicate that Kentucky grown hay is not a risk factor for MRLS. We can also conclude that producers of hay for consumption by pregnant mares should prevent their contamination with eastern tent caterpillar larvae.