

VETERINARY MEDICAL DIAGNOSTIC LABORATORY NITRATE-AFLATOXIN UPDATE (8/18/2012)

Nitrate Accumulation in Plants: Nitrate accumulation is a major concern in drought-damaged corn and species of *Sorghum*, as well as various noxious weeds growing in pastures. In addition to drought stress, high rates of fertilization, plant immaturity, and even excessive rain can influence nitrate concentrations in corn and other plants. Usually, nitrate concentrates in the bottom 12 to 18 inches of the stalk, but much of the drought-damaged corn currently being tested at the Veterinary Medical Diagnostic Laboratory (VMDL) at the University of Missouri, using the diphenylamine nitrate “spot” test, is showing high nitrate concentrations all the way up the stalk, without the usual decrease in nitrate content 18 to 24 inches above the base.

Potential Adverse Health Effects in Animals: Nitrate in forage→Nitrate in rumen→Nitrite in rumen→Nitrite in blood. Nitrite in the blood converts hemoglobin to methemoglobin, which doesn't bind oxygen. Methemoglobin formation is associated with “chocolate brown” blood, lethargy, and exercise intolerance, as well as, potentially, abortion and, even, sudden death in cattle and other susceptible ruminants.

“Spot” Tests: Preparations of diphenylamine in concentrated sulfuric acid can be obtained in person by MU Extension personnel from the VMDL, after completion of appropriate paperwork. Several cornstalks from the same field should be tested at 6 inch increments, starting at the base, until there is a dramatic decrease in the amount of nitrate present from one height to the next. “High” nitrate concentrations ($\geq 1\%$ nitrate) are indicated by a rapid (<20 seconds) change in color to dark blue. If fields differ in varieties of corn being grown, severity of drought damage, and/or amount of nitrogen fertilizer being applied/acre, more than one sample (sample = 3 to 5 plants) should be tested. “Spot” testing is available at the VMDL for \$16.25/sample of several stalks.

Quantitative Nitrate Analyses: The VMDL recommends that samples of corn or other plants with uniformly high nitrate concentrations throughout the stalks/stems, as determined by the “spot” test, be chopped up and submitted to the VMDL for quantitative nitrate analyses of the green chop. Quantitative nitrate analyses provide a more accurate estimate of the nitrate content in green chop, because it takes into account any dilution effects of leaves and ears, which are often lower in nitrate content than stalks or stems. Recently submitted samples of have contained quantitatively-determined nitrate concentrations anywhere from less than 0.1 to greater than 3% (<1,000 to >30,000 ppm or parts per million). These analyses cost \$23.00/sample (results in 72 to 96 hours). **Sample = 1 quart minimum/1 gallon maximum**

Interpretation of the Results: Accurate interpretation of the results of quantitative nitrate testing is best done in cooperation with extension personnel and veterinarians. Nitrate concentrations equal to or greater than 1% (10,000 ppm) on a dry-matter basis have been associated with acute death of cattle from nitrate/nitrite poisoning. In order to prevent nitrate-associated abortions, pregnant cattle should not be fed forage containing greater than 0.5% (5,000 ppm), with total dietary concentrations <0.25% (2,500 ppm).

Management: Green chop containing “safe” concentrations of nitrate should be fed as soon as is possible after chopping, and fed cattle (as opposed to hungry cattle waiting to be fed) should be adapted to this new forage, with gradual, supervised introduction of the green chop. Dilution of high-nitrate-containing forages with low-nitrate containing forages can decrease the overall nitrate concentration in feedstuffs. Ensiling high-nitrate containing forages is another means by which nitrate concentrations can be decreased in forage. Nitrate concentrations should be rechecked in mixed forage rations or ensiled forages prior to feeding. There are products containing “probiotics”, which reportedly decrease nitrite production in the rumen. The utility of such products for a given management system should be assessed in consultation with a veterinarian.

Herbicide Application to Alternative Forages: Other drought-damaged crops, such as soybeans, might be considered as additions or alternatives to feeding early harvested corn plants high in nitrate. However, herbicides are often applied to these other crops, and farmers, livestock producers, and veterinarians should be familiar with the manufacturer's label instructions and livestock withdrawal times pertaining to the use of treated crops for animal forages or bedding. It should also be noted that some herbicides can enhance the palatability of weeds, as well as the ability of some plants to accumulate nitrate.

HOW MUCH NITRATE IS TOO MUCH?

NO ₃ ⁻ -N	NO ₃ ⁻	Category	Recommendation
ppm			
0 to 550	0 to 2,500	SAFE	Forage is generally safe to feed to all classes of livestock.
550 to 1,100	2,500 to 5,000	CAUTION	Forage with this nitrate (NO ₃ ⁻) content can cause a problem with pregnant and young animals. Do not feed forage with nitrate levels this high in combination with non-protein nitrogen supplements, and limit forage with NO ₃ ⁻ levels this high to one-half of total ration.
1,100 to 3400	5,000 to 15,000	DANGER TOXIC	Limit forage with this NO ₃ ⁻ level to one-fourth of total ration. Should supplement forage of this type with energy, minerals and vitamin A.
More than 3,400	More than 15,000	EXTREMELY TOXIC	Forage with this NO ₃ ⁻ level or higher is toxic and should not be fed under any circumstance. If forage with this NO ₃ ⁻ concentration must be fed, it should be mixed with other feed and make up no more than 15 percent of the total ration.

AFLATOXINS IN CORN: Drought-damaged corn is highly susceptible to infection by *Aspergillus* fungi, and current as well as predicted weather conditions and storage of high-moisture corn at high temperatures facilitate the production of fungal toxins (mycotoxins) called aflatoxins, which can be passed into the milk of exposed dairy cattle at dietary concentrations greater than 20 ppb (parts per billion) or 0.02 ppm. At higher concentrations, aflatoxins can also cause serious illness, such as liver disease, in humans and animals, including cattle, sheep, goats, pigs, horses, birds, dogs, and cats. Recently submitted corn samples have contained elevated aflatoxin B₁ concentrations exceeding 100 ppb, which, under certain circumstances, can potentially be associated with clinical disease.

Sampling for Aflatoxin Analyses: Since “black lighting” is not a reliable method for ruling out aflatoxin contamination and aflatoxins can be produced in “pockets” of moldy corn, obtaining a representative sample for aflatoxin analysis is critical. Representative sampling can best be accomplished with input from MU Extension personnel but might consist of pooled samples of ears of corn or samples taken from a storage bin, truck, silo, or pit using a probe or moving stream collection techniques.

ELISA Analyses for Aflatoxin: Various commercially available ELISA kits and test strips can be used to screen for the presence of aflatoxins in feedstuffs, and these test kits and strips can be very useful for rapid field determinations of whether corn samples contain any aflatoxins or not.

Quantitative Aflatoxin Analyses: The VMDL recommends that representative samples of corn or corn-containing green chop, silages, or mixed feeds, especially those which have tested positive for aflatoxins using ELISA tests, be submitted to the VMDL for quantitative aflatoxin (aflatoxin B₁, B₂, G₁, G₂) analyses, using high performance liquid chromatography (HPLC). Analyses cost \$42.25/sample (results available in generally 3 to 5 working days). **Sample = 1 quart minimum**

Interpretation of the Results: Animals vary in their susceptibility to the adverse effects of aflatoxins, and accurate interpretation of analytical results should take into account animal species, age, and use, as well as published FDA action levels. Aflatoxin B₁ concentrations in feedstuffs equal to or greater than 20 ppb exceed FDA action levels for immature animals and some animal species, including dogs and cats, and can be associated with violative aflatoxin residues in the milk of exposed dairy cows.

Management: Dilution of aflatoxin-containing feedstuffs in the ration and ensiling these feedstuffs are ways by which dietary concentrations of aflatoxins can be reduced. Aflatoxin concentrations should be rechecked in mixed grain rations or ensiled forages prior to feeding. There are also “binders” which can, depending on the product, effectively decrease aflatoxin absorption, but use of such products should be discussed with a veterinarian and be based on clearly demonstrated aflatoxin binding efficacy in live animals.

VMDL Sample Submission: <http://vmdl.missouri.edu/> See: <http://extension.missouri.edu/index.aspx>

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