

## The Presence, Effect, and Diagnosis of Zearalenone in Dairy Cattle

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### Background

Mycotoxins are toxic substances that are produced by fungal species. These fungi can be found in a wide variety of plants and soil types. Toxigenic fungi are thought to be ubiquitous in the environment. Several characteristics are thought to be important for fungal growth, but the exact mechanisms of their proliferation are still unknown. The mycotoxins of major importance in Indiana are zearalenone, aflatoxin, DAS (vomitoxin) and fumonisin. Because the production of mycotoxins is strongly influenced by weather patterns and climates, the prevalence of each toxin varies with geographical location.

Zearalenone is naturally produced by the fungus *Fusarium roseum* and by some isolates of *Fusarium moniliforme*. Zearalenone is most commonly reported in the north central cornbelt of the United States and southeastern Canada. Zearalenone's estrogenic effects can be attributed to its complex chemical structure which makes it a phytoestrogenic molecule. Phytoestrogens are estrogenic compounds produced by plants/fungi. The fungus responsible for zearalenone production, *Fusarium spp.*, has also been shown to produce the nonestrogenic toxins deoxynivalenol and T-2 under appropriate conditions. Therefore, veterinarians and producers should be aware that mycotoxin contamination can be a multi-factorial problem.

### Clinical Effects

A wide variety of clinical effects attributed to zearalenone have been described in the literature. Decreased fertility, abnormal estrus cycles, swollen vulvas, vaginitis, reduced milk production and mammary gland enlargement are the most common findings reported in cattle and swine. From the aforementioned changes, single or multiple effects have been observed. A change in the estrus cycle can manifest itself in various forms. Prolonged, skipped, or irregular heats are commonly associated with zearalenone effects. While these abnormal estrus changes are not exclusively specific to zearalenone toxicity, one should investigate feed related causes when increases in abnormal estrus cycles are observed on farm.

In one report from a 150 cow dairy herd, an increased artificial insemination index (decreased fertility) was reported after the herd had been fed moldy hay. Zearalenone concentrations in the hay extracts were reported to be 14mg/kg of hay. Upon removal of the infected hay, the AI index returned to the previous level. A 20 cow Brown Swiss herd was observed showing anestrus, false estrus, or nymphomania with a gray vaginal discharge. Infectious causes were ruled out, and feed samples were taken. Zearalenone concentrations of 50 ppb and 100 ppb were detected in the corn silage and haylage,

respectively. Based on feed intakes, it was determined that these cows were receiving 1.6 mg of zearalenone per animal. Finally, an abnormal estrus cycle, nonresponsive to lutenizing hormone, was reported to have occurred in a dairy herd. Feed analysis yielded a zearalenone concentration of 25 mg/kg.

The clinical effects observed on heifers vary somewhat from cows. Mammary gland enlargement, swollen vulvas, and vaginitis are frequently observed more often in heifers as compared to cows. After being fed a ration containing moldy corn, 17 of 20 prepubertal dairy heifers developed enlarged mammary glands in at least one quarter. The secretion had a consistency of skim milk and appeared an off-white color. Zearalenone was determined to be present in the corn. Seven weeks after removal of the affected corn, all heifers were clinically normal. Weaver reported that when dietary zearalenone was greater than 12.5 ppm, a reduced conception rate was observed in virgin heifers.

Clinical effects of zearalenone vary by animal breed, age, and environment. While the majority of clinical symptoms observed mimic that of estrogenic stimulation, at the present time there is no way to determine what form of the syndrome will manifest in an affected group of animals. Multiple subclinical changes are probably occurring in affected animals. A combination of genetic and environmental factors most likely determines what outward clinical signs will be observed. Because the *Fusarium spp.* can produce estrogenic as well as nonestrogenic toxins, a variety of clinical signs are possible.

### **Feed Sampling and Diagnosis**

Diagnosis can be quite difficult and frustrating to the veterinarian and owner. Animals may have consumed the offending agent prior to the veterinary investigation, thus making collection of diagnostic samples difficult or impossible. This problem is further exacerbated by the sporadic and variable nature of the toxin. Zearalenone, as with other mycotoxins, can only be detected in feed or feed products. No individual animal serologic or tissue test, antemortem or postmortem, exists at this time. Histopathological changes indicative of mycotoxicosis can be observed in individual animal tissues; however, this is a subjective assessment and does not support a definitive diagnosis. Clinical signs, such as vaginal discharge or vulva enlargement, can serve to further support zearalenone toxicity, but this too is a nonspecific indication. Sampling feed or feed products is the only diagnostic tool available.

In order to maximize the probability of isolating zearalenone or any mycotoxin, the investigating veterinarian should utilize several steps to aid in his/her efforts. *Feed samples taken should reflect the feed/forage utilized during the time period of the problem.* This is not always possible, especially if low-level contamination is present. Clinical signs indicating a problem may not appear until weeks after the infected feed was consumed. *Samples should be representative of the entire product being fed.* Zearalenone levels can vary dramatically from areas in the same storage unit, or even among kernels on the same ear of corn! Even if moldy appearing areas are present, sampling of normal appearing feed (representative sample) should occur because normal appearing areas can be more severely affected. A good time to sample is after blending (such as auguring grain) has occurred. Periodic sampling of flowing grain/feed is recommended. Once individual stream samples have been collected, these can be combined and a subsample of at least 10 pounds should be submitted. *Samples should*

*be submitted dry or frozen and protected from light.* Heat, chemicals, and sunlight all have the potential to alter mold metabolites from their original structure and activity. Once samples have been frozen or refrigerated they should remain that way. *Submit individual feeds rather than mixed feeds if at all possible.* Isolation of toxins in mixed feed is often difficult because of the complex nature of mixed feeds. If individual components are not available, a list of ingredients should be supplied to the diagnostic lab. As with any other diagnostic test, quality sampling and sample submission is of the utmost importance.

Several testing options are available for the diagnosis of zearalenone. Thin Layer Chromatography (TLC) is one of the earliest and simplest analytical methods that has been developed. Because TLC does not require expensive analytical machines and is relatively uncomplicated, most laboratories can utilize TLC as a diagnostic tool. The Purdue ADDL utilizes this method for determination of mycotoxins. The process of TLC consists of extracting solvents, sample clean-up/purification, solvents for separation, and detection methods. Because of the reliability, cost effectiveness, and quick results, TLC is utilized in many diagnostic laboratories. A disadvantage of TLC is that the results are semi-quantitative.

High Performance Liquid Chromatography (HPLC) is another option for the diagnosis of mycotoxins. The sensitivity, accuracy, and quantification ability of this test has made HPLC popular in recent years. Cost of sampling is increased utilizing this method due to the expense of owning/operating the HPLC machine. HPLC samples also require extensive clean up, similar to TLC; however, an advantage is that quantitative, rather than semi-quantitative results, are obtained with HPLC.

### **Conclusion:**

Dairy cow and heifer productivity can be greatly altered by the presence of zearalenone in feedstuffs. Diagnosing and isolating zearalenone is often a difficult and frustrating problem. A basic knowledge of fungal dynamics, proper sampling techniques, sample handling, and persistence are important aids in the diagnosis of mycotoxicosis. Several testing modalities are available at various diagnostic laboratories. Diagnosis of zearalenone-induced estrogenic effects is based on history, clinical signs, and detection of zearalenone in feed. Treatment is based on removal of the contaminated feed and replacement with high-quality feedstuffs. While the incidence of zearalenone toxicity varies greatly, producers and veterinarians should be aware of this estrogenic substance and its effects on dairy reproductive health.

-by Christopher Witte, Class of 2003

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