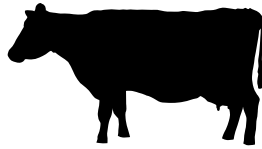




Mycotoxins and Dairy Cattle

A Review for Dairy Producers

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INTRODUCTION

Mycotoxins are ubiquitous and produced by several fungi, particularly by many species of *Aspergillus*, *Fusarium*, *Penicillium*, *Claviceps*, and *Alternaria* etc. They are secondary metabolites from fungi with unclear functions. Over 400 known mycotoxins have been identified today with a potential of 30,000 different metabolites. Among which aflatoxin, fumonisin, ochratoxin, T-2 toxin, vomitoxin, and zearalenone have the most attention by industry and the most research by academy.

In the early 1950's, death losses of cattle consuming moldy corn was reported in the United States (Sippel et al., 1953). Toxic substances from *Aspergillus* and *Penicillium* fungi were identified to cause the problem in later years (Burnside et al., 1957). The toxin responsible for the mortality was first purified by British scientists (Allcroft et al., 1961) from peanut meal originating in Brazil and was then named aflatoxin. Since then, many acute and chronic toxicity studies of aflatoxin and other mycotoxins have been demonstrated and reported.

With the ability of detoxification by rumen microorganisms, mycotoxins are considered less toxic in ruminants as compared to simple stomach animals. Among all of the mycotoxins, aflatoxin is the most problematic in dairy due to its derivative aflatoxin M₁ (AFM₁), present in milk, and its potential health hazard for human consumption. Aflatoxin can be present in several forms in feedstuffs, aflatoxin B₁, B₂, G₁, and G₂, with aflatoxin B₁ (AFB₁) being the most biologically active and toxic to animals (and humans).

MYCOTOXINS AND MILK

Aflatoxin B₁ and Aflatoxin M₁

Dairy cattle, in general, can tolerate a greater mycotoxin challenge. It has been hypothesized that the microbial population in the rumen is able to metabolize most mycotoxins. However, some of the toxic metabolites can be excreted in milk and cause public health concern. Among all known mycotoxins present in feed, aflatoxin (AFB₁) has the most significant impact to the dairy industry. Because little of the AFB₁ consumed is degraded by rumen and the resulting metabolite (aflatoxicol) is as toxic as AFB₁, it was suggested by Jouany and Diaz (2005) that ruminants have little protection against this toxin.

AFB₁ and AFM₁ (metabolite) are found in feeds and milk, respectively. Dairy cattle will produce milk contaminated with AFM₁ after consuming feeds contaminated with AFB₁. The AFB₁ is rapidly absorbed in the digestive tract and primarily metabolized by liver enzymes, converting it to AFM₁, which is then excreted in milk and urine. AFM₁ is less toxic than AFB₁; however, it has been demonstrated to be a carcinogen in rainbow trout (Sinnhuber et al., 1970) and causes morphological changes in rat liver (Pong and Wogan., 1971). The carcinogenic and highly toxic effects of aflatoxin and its metabolites has resulted in aflatoxin being highly regulated by most countries in the world (Table 1). Once it exceeds the regulatory limits, the AFM₁ contaminated milk, by law, has to be discarded to prevent it from getting back into the food chain. AFM₁ contamination in milk occurs often (not always above regulatory limits) because AFB₁ often occurs naturally in grains, by-products, and roughage.

Jouany and Diaz (2005) reported that the average transfer of AFB₁ in diet to AFM₁ in milk is 1.7%. With this figure, the authors calculated that only 30 ppb of AFB₁ in feed will result in 0.5 ppb AFM₁ (above regulatory limits in USA). Using the same calculations, in the EU, only 3 ppb dietary AFB₁ would result in milk being over the regulatory limits (0.05 ppb AFM₁). It is not practical to completely eliminate the use of AFB₁ contaminated feed ingredients; however, it is possible to control the toxin, preventing or at least reducing the concentration of AFM₁ in milk.

Ozonization and ammoniation have been shown as promising treatments for AFB₁ contaminated corn and cottonseed meal because it can be used in large batches of product (CAST, 2003). However, both methods are time consuming and economically impractical. Besides, ammonia treated grains are not currently allowed for interstate shipments in the U.S. Therefore, use of adsorbent materials in feeds to prevent toxin absorption by dairy cattle becomes a more feasible strategy. A comprehensive review of different types of mycotoxin adsorbents is reported by Huwig et al. (2001). Dairy producers are encouraged to read the paper for better understanding of mycotoxin binders including natural vs. synthetic adsorbents and biological methods, such as enzymes and yeasts.

TABLE 1: Global regulation of aflatoxin M₁ (AFM₁) in milk.

Countries	AFM ₁ Concentration in Milk
EU Countries	Less than 0.05 ppb
China, Japan, Mexico, Thailand, USA etc.	Less than 0.5 ppb
Nigeria	Less than 1.0 ppb

To control the amount of AFB₁ present in animal feeds and human foods, the U.S. Food and Drug Administration (FDA) has established ‘action levels’ for aflatoxin in feed ingredients (Table 2). The action level is the maximum amount of AFB₁ that can be present in animal feedstuffs to avoid toxins in meat, egg, and milk products intended for human consumption. The maximum allowance of aflatoxin in feed ingredients used in dairy feeds is 20 ppb, which is the same for young pigs and chicks. Lactating cows are as sensitive to aflatoxin as young animals; not for the toxicity of AFB₁ to the cow but because of the resulting AFM₁ in the milk.

TABLE 2: U.S. FDA action levels for aflatoxin in animal feeds.

Commodity	Action Levels, ppb
Corn, peanut meal, cottonseed meal, and other animal feeds ingredients intended for dairy animals, or when the intended use is not known	20
Corn, peanut meal, and other animal feeds and feed ingredients but excluding cottonseed meal, intended for immature animals	20
Corn and peanut meal intended for breeding beef cattle, breeding swine, or mature poultry	100
Corn and peanut meal intended for finishing swine of 100 pounds or greater	200
Corn and peanut meal intended for finishing (i.e. feedlot) beef cattle	300
Cottonseed meal intended for beef cattle, swine, or poultry (regardless of age or breeding status)	300

The appearance of AFM₁ levels, greater than 0.5 ppb, in milk can be found in as quickly as 4 hours after placing 13 mg AFB₁ directly into the rumen of a cow (Applebaum et al., 1982; Figure 1). Therefore, speed or rate of AFB₁ binding is a critically important attribute for dairy producers to consider when selecting a mycotoxin binder. A natural hydrated sodium calcium aluminosilicate (HSCAS) is superior to competitive products, such as enzymes and yeast cell wall, because of its binding efficiency speed.

Once AFB₁ is absorbed into the cow’s body, the clearance of AFM₁ in milk may take 5 to 7 days depending on the amount and duration of the AFB₁ consumption (Whitlow and Hagler, 2005). Diaz et al. (2004) conducted a trial and fed aflatoxin to lactating cattle with or without the addition of clay products (HSCAS) in feed (Figure 2). A surge of AFM₁ in milk was observed after AFB₁ added to feed; it declined gradually after AFB₁ removed from feed. Without a toxin absorbent in feed, the concentration of AFM₁ in milk was higher than the current U.S. regulation (0.5 ppb). On the contrary, with the addition of 1% clay product, AFM₁ in all sampled milk was under the U.S. regulation, whether AFB₁ was present or not.

FIGURE 1: Milk aflatoxin M₁ concentrations after placing aflatoxin B₁ in rumen.

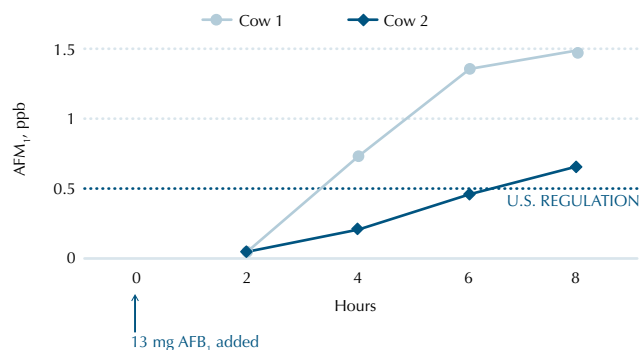
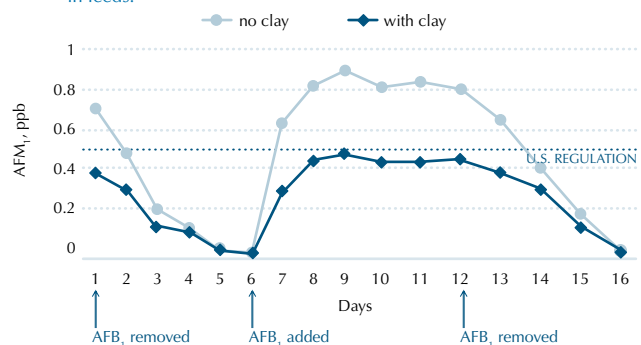


FIGURE 2: Concentration of aflatoxin M₁ in milk with or without 1% clay product in feeds.



Similar results were also found in dairy ewe (Battacone et al., 2003 and 2005). AFM₁ appeared in the sheep milk 6 hours after single AFB₁ dosage (2 mg), and then the concentration decreased with time. Trace amounts of AFM₁ was detectable until 78 hours after the dosage. In a separate study, the appearance of AFM₁ in sheep milk was shown to be AFB₁ dose dependent. When ewes were fed a diet containing 128 ppb AFB₁, the level of AFM₁ exceeded EU regulation (0.05 ppb) as early as 12 h post-treatment. When the ewes diet contained 64 ppb or 32 ppb AFB₁, the level of AFM₁ exceeded EU regulation at 24 hours and 144 hours post-treatment, respectively.

The stability of AFM₁ in milk, upon storage, has been studied; however contradicting results were reported depending on the method of processing. A report from U.S. FDA indicated that AFM₁ was stable for 18 days when milk was pasteurized and for 120 days when milk was frozen at -18°C (Stoloff et al., 1975). In the study, 2 ppb synthetic AFM₁ was added to raw milk, the raw milk was then divided into 2 halves. One half served as control and the other half pasteurized at 63°C for 30 min. Both halves were then stored at 4°C for 18 days. After 18 days, 100% of AFM₁ was recovered from samples with or without pasteurization (Table 3).

TABLE 3: Effect of pasteurization (63°C, 30 min) and refrigeration (4°C) on AFM₁ stability.

Treatments	AFM ₁ , µg/L	
	D-1	D-18
Raw milk	1.8	1.8
Pasteurized milk	1.7	1.8

In the second study, highly AFM₁ contaminated milk was obtained from a university research farm and was stored at -18°C (Stoloff et al., 1975). The concentration of AFM₁ in the samples was measured as storage time increased (Table 4). AFM₁ concentration in milk was stable for up to 2 months and started to decline after 68 days of storage. However, the concentration was still above 60% of initial concentration after 120 days of storage and considered not safe for consumption.

TABLE 4: Effect of frozen storage (-18°C) on AFM₁ stability.

	D-1	D-12	D-53	D-68	D-100	D-120
AFM ₁ , µg/kg	5.4	6.2	7.0	4.9	4.0	3.4

The presence of AFM₁ in milk is not the only problem resulting from dairy cows consuming aflatoxin contaminated feeds.

Studies have shown decreased milk production by feeding aflatoxin contaminated feed to dairy cows (Applebaum et al., 1981). In the study, 13 mg of unpurified aflatoxin was placed directly into the rumen of fistulated Holsteins for 7 days. Milk production was reduced significantly after impure AFB₁ was dosed directly into the rumen (Table 5). Reduced milk production was not a result of feed intake because total feed intake was not affected by AFB₁ presence. While feeding pure aflatoxin did not reduce milk production in the study, AFM₁ concentration in milk was significantly increased (P<0.05) with pure or impure aflatoxin.

TABLE 5: Milk production by feeding impure aflatoxin in cow.

Lactation (Month)	Milk Production, kg		Total Intake, (Concentrate + Hay, kg)	
	Before	After	Before	After
8	21.2	18.4	21.9	17.0
3	24.3	22.7	30.0	30.6
10	20.8	18.1	17.7	19.2
Ave	21.1 ^a	19.7 ^b	23.7	22.3

^{a, b} - significant difference between treatments (P<0.05)

Clinical symptoms of feeding AFB₁ contaminated feed to calves had been well documented (Lynch et al., 1970; CAST, 2003). Calves fed contaminated diets ranging from 0.8 to 8 ppm of AFB₁ for 6 weeks showed no difference in weight gain and feed intake. However, serum alkaline phosphatase increased when calves were fed diets that contained more than 2 ppm AFB₁. Histological examination confirmed the livers were enlarged with pale color in those treatments containing 2 ppm (0.02 mg/kg BW) and higher in feeds.

OTHER MYCOTOXINS AND MILK

Unlike AFM₁, no other mycotoxins are tightly regulated in milk. However, some toxins can be transferred into milk. In a study by Robinson et al. (1979), sows were fed 12 ppm T-2 toxin for 220 days and six days after parturition. A milk sample contained 76 ppb T-2 toxin. In a separate study, 182 mg of T-2 toxin was intubated into pregnant Holstein cow for 15 days. T-2 toxin was then found ranging from 10 to 160 ppb in milk (Robinson et al., 1979).

Unlike T-2 toxin, vomitoxin (deoxynivalenol, DON) was not detectable in milk when cows were fed 66 mg/kg for 5 days (Cote et al., 1986) or a single dose of 920 mg (Prelusky et

al., 1984) in dairy cattle. However, a less toxicity metabolite, de-epoxydeoxynivalenol (DOM-1; produced from DON by rumen microbes) was found at concentrations up to 26 ppb ($\mu\text{g/L}$) in milk. Although the significance of the presence of DOM-1 to public health is unknown, dairy producers should be more proactive in controlling mycotoxins to prevent unnecessary public concerns.

Non-lactating cows fed 6.4 mg/kg DON for 6 weeks showed no noticeable symptoms or illness as compared to the control (Trenholm et al., 1985; conducted by Canadian Agriculture Research Stations). The researchers also investigated the impact of DON on milk production. Results showed that feeding medium or high levels of DON (6 or 12 ppm, respectively) did not alter dry matter intake, milk production, or protein and lactose contents in the milk. However, lower milk fat content ($P < 0.05$) was found in medium DON fed cows, but not in high DON fed cows (Table 6). The authors could not explain why lower fat was observed in cows fed 6 ppm DON.

TABLE 6: Milk production and fat content after feeding different levels of DON.

Items	DON- 0 ppm	DON- 6 ppm	DON-12 ppm
DON intake, mg/d	0.6	42.7	104.2
DON intake, kg/d	16.3	15.9	16.3
Milk yield, kg/d	22.8	21.4	21.5
Milk fat, %	3.92 ^a	2.77 ^b	3.30 ^a
4% FCM, kg/d*	21.6	18.1	19.4

* Fat correct milk yield.

^{a, b} - significant difference between treatments ($P < 0.05$)

However, in an earlier study Noller et al. (1979) showed no influence of feeding DON on milk production and milk composition. In the study, a total of 54 lactating Holsteins were used and divided into 3 groups. Group one was fed a control diet with clean corn (20% of diet); group two fed diet with corn (20% of diet) contaminated with trichothecenes (DON 12-13 ppm and zearalenone 500 ppb in corn), and group three fed a diet with normal and contaminated corn at 50:50 mixture (10% of each in the diet). Results clearly indicated no difference of milk production or milk fat by feeding DON-ZEA contaminated feed, but the cows' weight gain was significantly reduced by consuming molded corn (Table 7).

TABLE 7: Effects of moldy corn (DON 12 ppm and ZEA 500 ppb) on lactating cows.

	Group I clean corn	Group II moldy corn 2.5 ppm DON 100 ppb ZEA	Group III 50 : 50 clean : moldy corn 1.25 ppm DON 50 ppb ZEA
DM intake, % live weight	2.90	2.79	2.85
Milk production, kg/d	22.7	23.2	22.9
Fat, %	3.81	3.82	3.81
Body weight gain, g/d	872 ^a	486 ^b	595 ^b

^{a, b} - significant difference between treatments ($P < 0.05$)

Moderate levels of fumonisin show no influence on milk yield; however, a high dosage of fumonisin for long period has detrimental effects on milk production. Dairy cattle (Holsteins and Jerseys) fed diets containing 100 ppm fumonisin from approximately 7 days prior to freshening through 70 days postpartum demonstrated lower milk production (approximately 9 kg a day at d-56) with an average of 6 kg difference per cow per day (Diaz et al., 2000).

TEST METHODS

There are simple, fast, semi-quantitative tests that can be used for AFM₁. Kits using ELISA (enzyme-linked immunosorbent assay) technology are available commercially for farm use. AFM₁ can also be analyzed using HPLC (high performance liquid chromatography), which is more of a quantitative analysis, but requires very expensive equipment, a clean analytical lab and an experienced technician. On a typical farm, samples would have to be sent out to an analytical lab for quantitative AFM₁ analysis.

CONCLUSION

Dairy cattle are less sensitive to mycotoxins as compared to poultry, swine, equine, and aquaculture species. However, the concentration of AFM₁ in milk is highly dependent upon dietary aflatoxin and the threat to humans makes aflatoxin in dairy feeds a constant concern. Because of the efficiency of AFB₁ conversion to AFM₁ in milk, only 30 ppb (3 ppb in EU) of aflatoxin contaminated feed is required to increase AFM₁ in milk, which could result in discarded milk and lost profit. Tolerance to fumonisin, ochratoxin, vomitoxin and

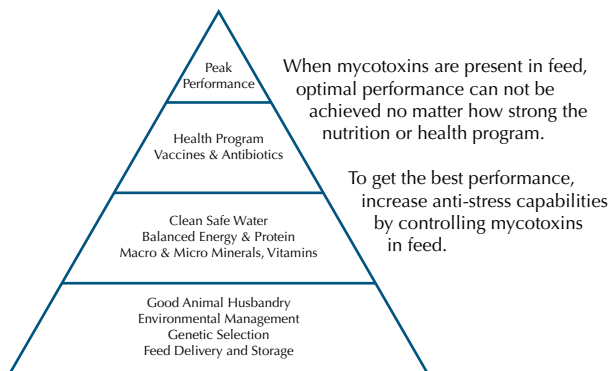
T-2 toxin are generally higher in dairy than simple stomach animals. Major toxins and their sensitivities in dairy cattle are summarized in the Table 8.

TABLE 8: Summary of mycotoxins sensitivities in dairy.

Mycotoxins	AFB ₁	DON	FUM	OTA	T-2	ZEA
Sensitivity	+++++	+++	+++	+	++	+/-
Toxin Tolerance	20-100s ppb	100s ppm	100s ppm	1000s ppm	500s ppm	Gender dependent

It is important to keep in mind that untreated mycotoxin contaminated feeds fed to dairy cattle may reduce milk production, alter milk compositions, or produce toxins in milk. No matter how strong the nutrition and health program, if dairies are not able to control mycotoxins, they will never achieve the greatest genetic potential from the animal and make the greatest profit. Therefore, controlling mycotoxins is the key in managing the peak performance of the dairy business (Figure 3).

FIGURE 3: Controlling mycotoxins is the key in managing animal peak performance.



REFERENCES

- Allcroft, R. R., B. A. Carnagham, K. Sargent, and J. O'Kelly. 1961. A toxic factor in Brazilian groundnut meal. *Vet. Rec.*73:428.
- Applebaum R. S., R. E. Brackett, D. W. Wiseman, E. H. Marth. 1982. Responses of dairy cows to dietary aflatoxin: Feed intake and yield, toxin content, and quality of milk of cows treated with pure and impure aflatoxin. *J Dairy Sci.* 65:1503-1508.
- Battacone, G., A. Nudda, A. Cannas, A. Cappio Borlino, G. Bombo, and G. Pulina. 2003. Excretion of aflatoxin M₁ of dairy ewes treated with different doses of aflatoxin B₁. *J Dairy Sci.* 86:2667-2675.
- Battacone, G., A. Nudda, M. Palomba, M. Pascale, P. Nicolussi, and G. Pulina. 2005. Transfer of aflatoxin B₁ from feed to milk and from milk to curd and whey in dairy sheep fed artificially contaminated concentrates. *J. Dairy Sci.* 88:3063-3069.
- Brunside, J. E., W. L. Sippel, J. Forgacs, W. T. Carll, M. B. Atwood and R. E. Doll. 1957. A disease of swine and cattle caused by eating moldy corn. II. Experimental production with pure cultures of molds. *Amer. J. Vet. Res.* 18:817.
- Bullerman, L. B. 1981. Public health significance of molds and mycotoxins in fermented dairy products. *J Dairy Sci.* 64:2439-2452.
- Chi, F. 2008a. Mycotoxins and poultry – a review for poultry producer. Oil-Dri Corporation of America, Animal Health and Nutrition Division Technical Bulletin.
- Chi, F. 2008b. Mycotoxins and swine – a review for swine producer. Oil-Dri Corporation of America, Animal Health and Nutrition Division Technical Bulletin.
- Cathey C. G., Z. G. Huang, A. B. Sarr, B. A. Clement, and T. D. Phillips. 1994. Development and evaluation of minocolumn assay for the detection of aflatoxin M₁ in milk. *J Dairy Sci.*, 77:1223-1231.
- Carlson, M. P., S. E. Ensley, R. J. Grant. 2002. Aflatoxin M₁ in milk. *NebFact*, Univ. of Nebraska, NF564.
- CAST. Mycotoxins – risks in plants, animal, and human systems. 2003. Ames, Iowa, USA.
- Charmley, E., H. L. Trenholm, B. K. Thompson, D. Vudathala, J. W. G. Nicholson, D. B. Prelusky, and L. L. Charmley. 1993. Influence of level of deoxynivalenol in the diet of dairy cows on feed intake, milk production, and its composition. *J. Dairy Sci.* 76:3580-3587.
- Cote, L. M., A. M. Dahlem, T. Yoshizawa, S. P. Swanson, and W. B. Buck. 1986. Excretion of deoxynivalenol and its metabolite in milk, urine, and feces of lactating dairy cows. *J Dairy Sci.* 69:2416-2423.
- Coulombe R. A. 1993. Symposium of biological action of mycotoxins: Biological action of mycotoxins. *J. Dairy Sci.* 76:880-891.
- Diaz, D. E., B. A. Hopkins, L. M. Leonard, W. M. Hagler, Jr., and L. W. Whitlow. 2000. Effect of fumonisin on lactating cattle. *J. Dairy Sci.* 83(abstr.):1171.
- Diaz, D. E., W. M. Hagler, Jr., J. T. Blackwelder, J. A. Eve, B. A. Hopkins, K. L. Anderson, E. T. Jones, and L. W. Whitlow. 2004. Aflatoxin binders II: reduction of aflatoxin M₁ in milk by sequestering agents of cows consuming aflatoxin in feed. *Mycopathologia* 157:233-241.
- Huwig A., S. Freimund, O. Kappeli, H. Dutler. 2001. Mycotoxin detoxification of animal feed by different adsorbents. *Toxicology Let.* 122:179-188.
- Jouany J.P. and D.E. Diaz. 2005. Effects of mycotoxins in ruminants. In: *The Mycotoxin Blue Book* (D.E. Diaz, ed). Nottingham University Press, Nottingham, UK. pp. 295-321.
- Lyncy G. P., G. C. Todd, W. T. Shalkop, and L. A. Moore. 1970. Responses of dairy calves to aflatoxin-contaminated feed. *J. Dairy Sci.* 53:63-71.
- Lynch, G. P. 1972. Mycotoxins in feedstuffs and their effect on dairy cattle. *J. Dairy Sci.*55:1243-1255.
- Marquardt R. R. and A. A. Frohlich, 1992. A review of recent advances in understanding ochratoxicosis. *J. Anim. Sci.* 70:3968-3988.
- Noller C. H., M. Stob, and J. Tutie. 1979. Effect of feeding *Gibberella zeae*-infected corn on feed intake, body weight gain, and milk production of dairy cows. *J Dairy Sci.* 62:1003-1006.

Pennington, J. A. 2005. Aflatoxin M₁ in milk. Univ. of Arkansas extension service.

Pong, R.S. and G. M. Wogan. 1971. Toxicity and biochemical and fine structural effects of synthetic aflatoxins M₁ and B₁ in rat liver. J. Natl. Cancer Inst. 47:585

Price, W. D., R. A. Lovell, and D. G. McChesney. 1993. Naturally occurring toxins in feedstuffs: Center for veterinary medicine perspective. J. Anim. Sci. 71:2556-2562.

Robinson T. S., C. J. Mirocha, H. J. Kurtz, J. C. Behrens, M. S. Chi, G. A. Weaver, and D. Nystrom. 1979. Transmission of T-2 toxin into bovine and porcine milk. J. Dairy Sci. 62:637-641.

Sharma R. P. 1993. Immunotoxicity of mycotoxins. 1993. J Dairy Sci. 76:892-897.

Sinnhuber, R. O., D., J. Lee, J. H. Wales, M. K. Landers, and A. C. Keye. 1970. Aflatoxin M₁, a potent liver carcinogen for rainbow trout. Fed. Proc. 29, Abstr. 1800.

Sippel, W. L., J. E. Brunside, and M. A. Atwood, 1953. A disease of swine and cattle caused by eating moldy corn. Proceedings Book, Vet. Med. Assoc., 19th Annual Meeting. 174:181.

Stoloff, L., M. Truchsess, N. Hardin, O. J. Francis, J. R. Hayes, C. E. Polan, and T. C. Campell. 1975. Stability of aflatoxin M in milk. J. Dairy Sci. 58:1789-1793.

Trenholm, H. L., B. K. Thompson, K. E. Hartin, R. Breenhalgh, and A. J. McAllister. 1985. Ingestion of vomitoxin (deoxynivakenol)-contaminated wheat by nonlactation dairy cows. J Dairy Sci. 68:1000-1005.

Whitlow L. W. and W. M. Hagler, Jr. 2005. Mycotoxins in dairy cattle: occurrence, toxicity, prevention and treatment. Proc. Southwest Nutr. Conf.:124-138.

Wood, G. E. 1992. Mycotoxins in foods and feeds in the United States. J Anim. Sci. 70:3941-3949.



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