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UNIVERSITY OF MINNESOTA

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VETERINARY CONTINUING EDUCATION



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Laboratory Analysis of Mycotoxins

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1. TEST KITS:

AOAC-RI: Performance Tested Methods™ - Certified Test Kits

Test Kit Name	Manufacturer	Validated	
		Analytes	Matrixes
AflaTest ^R P	Vicam L. P.	aflatoxin residues	grain & grain products
Veratox ^R AST	Neogen Corporation	aflatoxin residues	grain & grain products, cottonseed, cottonseed meal, raw peanut & roasted peanuts
Veratox ^R for Vomitoxin	Neogen Corporation	deoxynivalenol (DON or vomitoxin)	corn, corn meal, corn screenings, wheat, wheat midds, wheat flour, wheat bran & barley

2. SUPPLEMENTAL TESTS:

AOAC-RI: Methods™ - (Analytical Chemistry Methods)

AFLATOXIN

AOAC Number & Title				
	Method	Sensitivity	Matrix	Type
	Reference:			
1.	975.36: Aflatoxins in Foods and Feeds			
	Romer Minicolumn Method	≥ 5 ppb	almonds	Qualitative
		≥ 10 ppb	white & yellow corn, peanut & cottonseed meals, peanuts, peanut butter, pistacio nuts.	
		≥ 15 ppb	mixed feeds	
	References:	<i>JAOAC</i> : 58, 500(1975); 59, 110(1976); 61, 340(1978); 62, 136 (1979).		
2.	979.18: Aflatoxins in Corn and Peanuts			
	Holaday-Velasco Minicolumn	≥ 10 ppb	white & yellow corn, raw, shelled peanuts	Qualitative
	References:	<i>JAOAC</i> : 62, 1070(1979); 64, 674(1981).		
3.	968.22: Aflatoxins in Peanuts and Peanut Products			
	CB Method	TLC		Qualitative
	References:	<i>JAOAC</i> : 51, 67(1968); 53, 104(1970); 57, 871(1974); 71, 70(1988).		
4.	970.45: Alfatoxins in Peanuts and Peanut Products			
	BF Method	TLC		Qualitative
	References:	<i>JAOAC</i> : 53, 104(1970); 57, 87(1974); 66, 355(1983).		

5.	971.23: Aflatoxins in Cocoa Beans		
	Modification of CB Method	TLC	
	References:	<i>JAOAC</i> : 52, 72(1969); 54, 540(1971).	
6.	971.24: Aflatoxins in Coconut, Copra and Copra Meal		
	Thin Layer Chromatographic Method	TLC	
	References:	<i>JAOAC</i> : 54, 874(1971).	
7.	972.26: Aflatoxins in Corn		
	Thin Layer Chromatographic Method	TLC	
	References:	<i>JAOAC</i> : 55, 781(1972).	
8.	980.20: Aflatoxins in Cottonseed Products		
	Thin Layer and Liquid Chromatographic Methods	TLC Quantitative TLC HPLC	
	References:	<i>JAOAC</i> : 63, 899(1980); 66, 418(1983); 69, 240, 294(1986).	
9.	989.06: Aflatoxin B₁ in Cottonseed Products and Mixed Feed		
	Enzyme-Linked Immunosorbent Screening Method	Screening aflatoxin B ₁ at \geq 15 ng/g (ppb)	
	References:	<i>JAOAC</i> : 72, 326(1989).	
10.	970.46: Aflatoxins in Green Coffee		
11.	978.15: Aflatoxin B₁ in Eggs		

12.	985.17:	Identification of Aflatoxin B₁		
	Confirmation Method	TLC cleanup Mass Spectrometry		
	References:	<i>JAOAC</i> : 63, 1052(1980); 68, 636 (1985).		
13.	970.48:	Toxicity of Aflatoxin B₁ - Chicken Embryo Bioassay.		
14.	974.17:	Aflatoxin M₁ in Dairy Products - Thin Layer Chromatographic Method		
		TLC	Fluid milk Powdered milk Blue cheese Ricotta cheese Cheddar cheese Butter	
	References:	<i>JAOAC</i> : 56, 1431(1973); 57, 847, 852(1974); 63, 247(1980).		
15.	980.21:	Aflatoxin M₁ in Milk and Cheese - Thin Layer Chromatographic Method		
		TLC Confirmation by derivative formation on TLC plate		
	References:	<i>JAOAC</i> : 61, 809(1978); 63, 907(1980) <i>J. Am. Oil Chem. Soc.</i> 56, 800(1979).		
16.	982.24:	Aflatoxins B₁ and M₁ in Liver - Thin Layer Chromatographic Method		
17.	982.25:	Aflatoxins B₁ and M₁ in Liver - Confirmation Method (Two dimensional TLC)		
18.	986.16:	Aflatoxins B₁ and M₁ in Liver - Confirmation Method (HPLC)		

DEOXYNIVALENOL

AOAC Number:		Title			
	Method	Sensitivity	Commodity	Type	
1.	986.17:	Deoxynivalenol in Wheat			
	TLC	≥ 300 ng/g	wheat	Semi-quantitative	
	Reference:	<i>JAOAC</i> : 69, 37(1986).			
2.	986.18:	Deoxynivalenol in Wheat			
	Gas Chromatography			Quantitative	
	Reference:	<i>JAOAC</i> : 69, 899(1986).			

OCHRATOXINS

AOAC Number & Title		Method	Sensitivity	Commodity	Type
1.	973.37:	Ochratoxins in Barley			
		TLC Confirmation by ethyl ester formation			Semi-quantitative
	References:	<i>JAOAC</i> : 56, 817, 822(1973).			
2.	975.38:	Ochratoxin A in Green Coffee			

PATULIN

AOAC Number & Title					
		Method	Sensitivity	Commodity	Type
1.	974.18:	Patulin in Apple Juice			
		TLC			Qualitative
	References:	<i>JAOAC</i> : 57, 62(1974).			

STERIGMATOCYSTIN

Mycotoxin	AOAC Number	Method	Sensitivity	Commodity	Type
	973.38:	Sterigmatocystin in Barley and Wheat - Thin Layer Chromatographic Method			
		TLC Confirmation by derivative formation			Semi-quantitative
	References:	<i>JAOAC</i> : 54, 86(1971); 56, 1123(1973).			

ZEARALENONE

AOAC Number					
		Method			Type
1.	976.22:	Zearalenone in Corn			
		TLC		Corn	Semi-quantitative
	Reference:	<i>JAOAC</i> : 59, 666(1976).			
2.	985.18:	alpha-Zearalenol and Zearalenone in Corn			
		HPLC			Quantitative
	Reference:	<i>JAOAC</i> : 68, 958(1985).			

2. SUPPLEMENTAL TESTS:

Summary: No analytical method has been validated for quantitation of aflatoxin, ochratoxin, deoxynivalenol, fumonisin or zearalenone in fermented feeds (corn silage, high moisture corn, haylage).

A common analytical approach:

Screen with one technique then confirm with a second technique.

Screening or qualitative tests: ELISA, TLC

Quantitative or confirmatory tests: HPLC, GC/MS

A. Screening Tests:

Aflatoxin	ELISA, TLC
Deoxynivalenol	ELISA, TLC
Fumonisin	ELISA, TLC
Ochratoxin	TLC
Zearalenone	TLC

Cost: \$ 15 to 50

Time: 1 day to 1 week

B. Confirmatory Tests:

Aflatoxin	HPLC
Deoxynivalenol	Gas Chromatography - Mass Spectroscopy
Premier Laboratory:	Howard Casper
	North Dakota State University
Fumonisin	HPLC
Zearalenone	HPLC

Cost: \$ 25 to 100

Time: 1 week

III. INTERPRETATION OF TEST RESULTS:

A. The test result itself:

1. Screening or Qualitative result.
2. Confirmatory result.
3. Quantitative result: How much is really there ?

Conclusion: **Qualitative results need to be confirmed.**

B. The Sample:

1. Field Fungi: Was it uniform across the field in the first place ?
Fusarium sp: Moisture (Wet years) Root rot/Stalk rot
 Hail Damage "Pink" mold

Aspergillus sp: Drought Stress
2. Does the sample represent one commodity or the finished ration ?
e.g Cottonseed: Aflatoxin source

Conclusion: **Need to determine actual exposure to the individual animal or the herd.**

C. The Animal:

1. **Aflatoxin:**

a. FDA - Regulatory Action Guidance:

Section. 683.100: Action Levels for Aflatoxins in Animal Feeds
(CPG 7126.33)

<u>Concentration</u>	<u>Matrix</u>	<u>Animal Intended for</u>
300 ppb	Corn Peanut products	Finishing (feedlot) beef cattle
	Cottonseed meal	Beef cattle Swine Poultry
200 ppb	Corn Peanut products	Finishing swine weighing 100 pounds or greater
100 ppb	Corn Peanut products	Breeding beef cattle Breeding Swine Mature Poultry
20 ppb	Corn Peanut products Other feeds	Immature animals
	Cottonseed meal	Dairy animals Animals species not specified above

2. Deoxynivalenol:

a. Food and Drug "Advisory Concentrations" Center for Veterinary Medicine

Species	Advisory Concentration
Swine Feed	5 ppm not to exceed 20 % of the ration.
Poultry or Ruminant Feed	10 ppm not to exceed 50 % of the ration.

b. Literature:

Dairy

1. To measure the effect of deoxynivalenol [vomitoxin] (DON) on intake of cows with a high milk yield. 4 multiparous and 4 primiparous Holstein cows in early lactation were fed on mixtures of clean and contaminated barley containing DON 0, 6, 18 and 24 mg/kg, for 3 weeks. Results suggest that diets providing DON up to 14.6 mg/kg concentrate or 31 mg/100 kg body weight, for high milk yield, did not affect feed intake, milk yield and composition or rumen pH over a 3-week period. However, caution is suggested as DON-contaminated barley can at times contain other mycotoxins.

Ingalls JR, Blair R. Influence of deoxynivalenol on feed consumption by dairy cows. 15th Western Nutrition Conference and the Canola Pre-Conference Seminar, Winnipeg, Manitoba, Canada, 13-14 September 1994. *Animal Feed Science and Technology*. 1996, 60: 3-4, 297-300.

2. 18 primiparous Holstein cows were used in a 10-week lactation study, preceded by a 2-week covariate period, to determine the effect of concentration of deoxynivalenol [vomitoxin] in the diet on cow performance and transfer of deoxynivalenol and its metabolite, deepoxydeoxynivalenol, to milk. Diets were formulated to contain deoxynivalenol at 0.6 and 12 mg/kg of concentrate DM, and daily intake of deoxynivalenol was 0.59, 42 and 104 mg respectively. Increasing deoxynivalenol in the diet did not affect intake of concentrate or forage. Total milk output was not affected; however, milk fat responded quadratically; cows given deoxynivalenol at 6 mg/kg of concentrate DM had the lowest milk fat content and fat output. Overall energetic efficiency was not influenced because reduced energy output in milk was compensated by increased body weight gains. No transfer of deoxynivalenol or deepoxydeoxynivalenol to milk was observed; concentrations were below detectable limits (1 µg/ml) using HPLC-MS. It was concluded that diets containing deoxynivalenol up to 6 mg/kg of dietary DM did not reduce feed intake of cows in this study and that deoxynivalenol or deepoxydeoxynivalenol was not transferred to milk. Further studies are required to confirm the apparent lack of effect of

deoxynivalenol on milk production.

Charmley E, Trenholm HL, Thompson BK, Vudathala D, Nicholson JWG, Prelusky DB, Charmley LL. Influence of level of deoxynivalenol in the diet of dairy cows on feed intake, milk production, and its composition. *J Dairy Sci.* 1993, 76: 11, 3580-3587.

3. Of concentrates from 100 farms 56 contained aflatoxin (37), deoxynivalenol (DON, 26) or zearalenone (1). Decline in rolling herd av. milk yield in summer or through the year was related to concentrate DON concn. ($P < 0.05$). Neither production nor health were related to aflatoxin concn., which were ≤ 70 $\mu\text{g}/\text{kg}$.

Whitlow LW, Nebel RL, Behlow RF, Hagler WM, Brownie CFG. Mycotoxins in North Carolina dairy feeds - a survey of 100 dairy farms. *J Dairy Sci.* 1986, 69: Suppl. 1, 223.

Beef

1. One hundred and eighty steers were fed rations containing 0, 8, 15 and 26 ppm DON throughout the feeding period (~ 150 days). Adverse effects on feed intake, feed to gain ratios, gain, serum biochemistry parameters, complete blood counts, carcass quality or rib steak palatability were not observed.

Dicostanzo A, Johnston L, Epley R, Meiske J, Chester-Jones H, Windels H, Casper H, Murphy M. Is deoxynivalenol contaminated barley associated with adverse health, production, or food safety effects in feedlot steers? *J An Sci* - Submitted.

3. Zearalenone:

Doses approximating 35 ppm zearalenone in feed when delivered for 2 heat cycles were not associated with adverse reproductive effects in adult dairy cattle.

1. Doses of 99% pure zearalenone (31.25, 62.5, 125, 250 or 500 mg) in gelatin capsules were given once a day orally to 18 nonpregnant, nonlactating, multiparous dairy cows for 2 consecutive oestrous cycles. There was no effect on serum progesterone concentrations, RBC, WBC, PCV, haemoglobin, and oestrous cycle length. Differential cell counts, clinical health, and sexual behaviour were not affected by the zearalenone. One cow from each of the groups given zearalenone and a control were killed at the end of the study. Zearalenone had no effect on the terminal bone marrow smears and did not induce any gross lesions discernible at PM examination or any microscopic lesions in representative samples of 30 tissues/cow. Rectal palpation of the reproductive tracts once a week indicated that the corpora lutea were small in cows given zearalenone. There was a general trend to increased haemoglobin concentrations in cows given the larger doses. Zearalenone of and by itself does not seem to be an important factor in dairy cow health.

Weaver GA, Kurtz HJ, Behrens JC, Robison TS, Seguin BE, Bates FY, Mirocha CJ. Effect of zearalenone on dairy cows. *Am J Vet Res.* 1986, 47: 8, 1826-1828.

2. Eighteen cyclic, virgin, Holstein heifers were given daily 250 mg of 99% purified zearalenone in a gelatin capsule orally, and 18 controls were given an empty gelatin capsule once a day. The study lasted through one non-breeding oestrous cycle and the next 2 consecutive oestrous cycles during which the 36 heifers were inseminated. The treated and control heifers had conception rates of 62% and 87%, respectively. There was no effect on the serum concentration of progesterone or the complete blood cell counts. Three heifers, bred but not pregnant by the end of the study, were killed and examined PM. The treated heifer did not have any zearalenone-attributable lesions, and there was no effect seen in the bone marrow smears. The remaining 33 heifers were sold as a herd, and the 31 pregnant heifers calved normally. There was no effect on the sex ratio of the offspring, which were all clinically healthy. Zearalenone lowered the conception rate of the treated heifers.

Weaver GA, Kurtz HJ, Behrens JC, Robison TS, Seguin BE, Bates FY, Mirocha CJ. Effect of zearalenone on the fertility of virgin dairy heifers. *Am J Vet Res.* 1986, 47: 6, 1395-1397.

3. Prelusky DB, Scott PM, Trenholm HL, Lawrence GA. Minimal transmission of zearalenone to milk of dairy cows. *J Environmental Science-and-Health.-Part-B,-Pesticides,-Food-Contaminants,-and-Agricultural-Wastes.* 1990, 25: 1, 87-103.
4. An outbreak of zearalenone poisoning, causing impaired fertility in 11 of 61 heifers, is reported from a farm in New Zealand in Feb. 1993.
Webber W. Zearalenone? Proceedings of the 11th seminar for the Society of Dairy Cattle Veterinarians of the New Zealand Veterinary Association held at Queenstown, New Zealand, 20-24 June, 1994. 1994, 166-171.
5. High-moisture maize was treated with a propionic acid preservative and stored in a 40 000 bushel steel bin. This maize heated and spoiled in storage and subsequently was retreated with the preservative. The out-of-condition maize was used as an ingredient in the ration for a dairy herd of cows and replacement heifers. The finished feed was cultured for fungi and assayed for mycotoxins. Results were 750 000 *Fusarium* spp. colonies/g of feed and 1.5 mg zearalenone and 1.0 mg deoxynivalenol [vomitoxin]/kg of feed. *Candida*, *Mucor* and *Penicillium* spp. and aflatoxin were also detected in the feed. Frequent episodes of behavioural oestrus of 2-5 d duration, that were not synchronized with the ovarian cycle, were observed. Cows in the second and third trimester of pregnancy also had episodes of behavioural oestrus. Idiopathic vaginitis was diagnosed. Mammary development occurred in the prepubertal heifers. Cows bred in true oestrus were found in true oestrus 35-55 d later. All of the heifers with precocious mammary development were subsequently culled from the herd because of sterility.
Coppock RW, Mostrom MS, Sparling CG, Jacobsen B, Ross SC. Apparent zearalenone intoxication in a dairy herd from feeding spoiled acid-treated corn. *Vet Hum Toxicol.* 1990, 32: 3, 246-248.

4. **Fumonisin:**

Fumonisin may become a significant mycotoxin from a human health/ regulatory standpoint. Studies reported thus far have not detected fumonisins in the milk of cattle dosed orally.

IV. MISCELLANEOUS TOPICS

a. Cystic ovaries

Concerns of reduced conception, enlarged vulva or udder.

Corn or corn silage: Consider zearalenone.

Legumes: Consider phytoestrogens.

b. Mold counts

1. Often < 10,000 cfu/g
2. When > 1,000,000 cfu/g often visibly moldy.

Potential undesirable effects:

1. Inhalation of spores.
2. Alteration of rumen microflora.
3. Reduced nutritive value.
4. Mycotoxins.

c. Microbe ID

Organism	ID	Adverse chemical(s)
Bacteria	<i>Listeria</i> sp	
	<i>Clostridium</i> sp.	
Yeast		
Molds	<i>Cladosporem</i> sp	None
	<i>Mucor</i> sp	None
	<i>Rhizopus</i> sp	
	<i>Penicillium</i> sp	Penicillic acid, patulin, PR toxin, roqufortine,
	<i>Fusarium</i> sp	DON, fumonisin, zearalenone
	<i>Aspergillus</i> sp	Aflatoxin

IV. CONCLUSIONS:

1. Controlled studies have not demonstrated a concentration of deoxynivalenol which induces adverse health or production effects in cattle.
2. A screening or qualitative test result (ELISA or TLC) should be confirmed by quantitative testing prior to implementing a change to the dairy feeding program.
3. Stored feeds that are visibly damaged may have reduced nutritional value.
4. Stored feeds that are visibly moldy may have more than 1 million colony forming units/gram of mold growth on them. Such feed may have amounts of yeast, bacteria (including *Clostridium* sp, & *Listeria* sp), molds (*Penicillium* sp) that do not support profitable milk production.
5. Proper corn silage and high moisture corn management reduces the risk from aerobic activity on stored feeds.
6. Ideally the best way to evaluate infestation of corn silage of high moisture corn with field fungi (*Aspergillus* sp, *Fusarium* sp) is when the crop is in the field.

Field Conditions

Look for:

wet areas of the field

root rot

stalk rot

drought

hail damage

"Pink" mold on ears

Faced with the choice between changing one's mind and proving there is no need to do so, almost everyone gets busy on the proof.

- Galbraith's Law.