

Detoxifying Aflatoxin Contaminated Cottonseed
Otis G. Lough, Agricultural Agent/Dairy
University of Arizona
4341 East Broadway, Phoenix, AZ 85040

Introduction

Aflatoxin, exceeding the 0.5 p.p.b. FDA actionable level, was detected in raw and processed milk supplies during August 1978 when a surveillance program was instituted by health authorities due to the wide spread marketing and feeding of a lot of highly contaminated cottonseed.

The problem was quickly corrected by the United Dairymen of Arizona, Inc., a co-op marketing agency representing 85-90% of Arizona's raw milk supply. The co-op banned the feeding of all cottonseed products by members and instituted a severe financial penalty program. This action removed vital feedstuffs from use which give Arizona dairymen a definite economic advantage in milk production.

The author instituted a series of investigations in cooperation with the United Dairymen of Ariz., dairyman, Conrad Gingg, Dr. Ralph Price, and others. The goal was to develop a practical method for reducing detectable levels in milk.

Background

Aflatoxin is a potent cancer causing agent in many animal species when fed at comparatively low levels over an extended period of time. At higher levels it can cause more immediate health problems. This is the basis for concern by both animal and human health authorities as more and more evidence indicates the occasional occurrence of aflatoxin on feeds and foodstuffs.

Peanuts, corn and cottonseed are the most commonly contaminated materials although nuts, cereal grains and many other materials have shown some aflatoxin contamination. The concern over corn is obvious due to it's wide spread use for both animal feed and human food. Cottonseed use is more regional, but in the southwest it has earned a reputation as an excellent feed addition, especially for high producing cows.

Aflatoxin Source

The common mold Aspergillus flavus is the most common producer of aflatoxin. It favors hot, humid environments similar to the irrigated areas of the southwest and the moist southeastern United States. However, favorable conditions may occur during the summer months in most all areas of the United States. The mold grows best where crops are stressed by insect damage, drought and other adverse growing conditions. It does not compete well under conditions where many mold species can grow, i.e. silage surfaces, manure, etc.

Aflatoxin - A Legal Hazard

The Food and Drug Administration has set actionable levels for aflatoxin at 20 p.p.b. in most foods and feedstuffs. The one exception is milk, where the actionable level is 0.5 p.p.b. Aflatoxin levels exceeding 20 p.p.b. in the total ration of dairy cows can result in milk exceeding the 0.5 p.p.b. actionable level. Total rations containing less than 15 p.p.b. will result in non-detectable levels in milk using the current testing methods (1979).

Most state health authorities, dairy co-ops and milk handlers have adopted the FDA standards. Therefore, debating the human health hazard of 0.5 p.p.b. in milk is an exercise in futility from the practical or economic sense. Milk exceeding the 0.5 p.p.b. level can result in direct economic loss. If co-mingled with other producer milk, it could be grounds for legal action by other producers and consumers who claim loss due to the initial contamination. Obviously, the good dairy manager will avoid producing milk that will exceed the actionable level of 0.5 p.p.b. Also, fortunately, the cow will usually be free of aflatoxin 96 hours (4 days) after being placed on clean feed.

Aflatoxin - An Animal Hazard

The first noticeable effects of aflatoxin contamination of dairy feedstuffs is decreased feed intake with the resulting drop in production. However, this is an insidious situation due to borderline effects that tend to be masked by individual cow susceptibility and many other conditions. Aflatoxin is associated with protein metabolism so it is wise to increase the protein level 1 to 2 percent if aflatoxin contamination cannot be completely eliminated. Also increasing the energy level and vitamin content, especially A, D, E, and K, is recommended.

Heat stress results in the animal being less able to detoxify aflatoxin. This further complicated summer feeding programs. Also, a most disturbing effect of aflatoxin is that it interferes with the animals' ability to develop immunity to disease. This increases disease problems and could render vaccination programs ineffective.

Inactivating Aflatoxin - Ammoniation

U.S.D.A. Agricultural Research Service workers at the New Orleans Southern Regional Research Laboratory successfully inactivated aflatoxin in peanut and cottonseed meals. They used an "autoclave" method for applying ammonia under pressure up to 50 p.s.i. for 30 minutes. This process has been commercially used to treat cottonseed at two oil seed mills in Arizona and one whole cottonseed treatment plant in California. Air pollution from released ammonia has been a continuing problem.

Other U.S.D.A. - A.R.S. workers at the Peoria, Illinois Field Station developed a method for inactivating aflatoxin contaminated corn at atmospheric pressure and ambient temperatures. They circulated ammonia through ducts under a pile of high-moisture corn (15-25%), sealed in plastic for 24 to 48 hours. The corn was kept sealed for 2 weeks before being opened and fed.

The author and associates at the University of Arizona conducted a "smallbag" laboratory trial in the fall of 1978 during the "Arizona Aflatoxin Crisis". 1 1/2% ammonia and 12% water was added to the contaminated cottonseed which was sealed in plastic bags and placed in direct sunlight. In 6 days, aflatoxin levels had dropped from the 1500-1900 p.p.b. range to 78 p.p.b. and in 21 days, to 53 p.p.b.

A field scale trial was then conducted using an Ag-Bagger machine and an ammonia applicator designed for silage making. Conrad Gingg, Tolleson, Arizona dairyman supplied the cottonseed and a string of 90 representative cows. Special funding was supplied by the University of Arizona, United Dairymen of Arizona and Maricopa County Farm Bureau. Four lots of seed were fed to the 90 cow string. Seed fed in the first 3 treatments had tested in the 2000 to 7000 p.p.b. range during August through October period in 1978. However, due to heating and enzyme activity, aflatoxin levels appeared to have dropped significantly by late December.

Ammoniated seed analyzing 650 p.p.b. pre-treatment was fed (7.25 pounds per cow per day) from day 2 through day 14. Ammoniated seed analyzing 538 p.p.b. pre-treatment was fed on day 15 through day 20. Untreated seed analyzing 320 p.p.b. was fed from day 21 through day 26. Ammoniated seed analyzing from 0 to 114 p.p.b. pre-treatment was fed from day 27 through day 30.

Aflatoxin averages in bulk milk adjusted for a one day carry over were:

- Pre-trial, .10 p.p.b.
- NH₃ - 650 treatment averaged .13 p.p.b. peaking at .18 p.p.b.
- NH₃ - 538 treatment averaged .14 p.p.b. peaking at .16 p.p.b.
- Untreated - 320 averaged .45 p.p.b. peaking at .55 p.p.b.
- NH₃ - 0-114 treatment dropped sharply from the .55 p.p.b. peak of the untreated seed to .09 p.p.b. on day 4. (See Figure 1)

Individual cow samples were taken of one milking at the end of each treatment period. Thirty-one cows were selected initially on a randomized basis for milk analysis. The simple average of individual cow analysis agreed closely with bulk tank analysis of the same day except for the pre-trial sampling. (See below)

	Date: 2/1	2/10	2/20	2/26	3/12
Bulk Sample (p.p.b.)	.10	.18	.11	.52	.09 (3/13)
Individual cow sample average (p.p.b.)	Trace	.15	.12	.56	.10

A companion feeding trial, using the 650 p.p.b. pre-treatment seed and untreated seed from the same lot, was conducted by Price and Brown of the University of Arizona.

Cows were fed 5 pounds of treated and untreated cottonseed for 8 days. Figure 2 shows that aflatoxin milk levels peaked one day after seed was removed from the ration and dropped off rapidly. It was gone completely by the 5th day following seed removal. Treated seed never reached the F.D.A. actionable level of 0.5 p.p.b., staying under 0.2 p.p.b. Untreated seed rose steadily peaking at 1.75 p.p.b. the day following seed removal. This test gave added support to the effectiveness of the bagging process for inactivating aflatoxin in cottonseed. (Figure 2)

Custom Field Application

Following the successful Gingg trial, three custom operators secured equipment to treat cottonseed on the farm. The Ag-Bagger machine and the Silopresse machine appear to work equally well. To date (June, 1979), approximately 6000 tons of cottonseed has been treated and bagged at some 50 different dairy operations.

Two operators are using batch-mixed 12% aqua-ammonia, which is applied through a perforated pipe located just above the throat of the bagging machine. A third operator uses a mixing tank to combine anhydrous ammonia and water at the treatment site. There have been some minor problems with even ammonia distribution primarily with the latter applicator. These appear to have been man-caused rather than mechanical.

The seed to be fed within the next 24 to 48 hours is exposed to air by cutting away the plastic.

No palatability problems have been observed. Also, no complaints concerning air pollution have been filed as only a small amount of ammonia is released at any one time.

No controlled feeding trials have been conducted to date. Performance of the treated seed in dairy rations appears to be very similar to untreated seed, however.

Initially the use of black plastic resulted in broken bags as the plastic heated and weakened from the sun's rays. The black bags were painted with white latex paint which solved the problem. Now however, white plastic or black inner liner with a white outside cover are being used for bagging.

Custom charges have varied from \$15 to \$20 per ton.

Recommended Treatment

Additional laboratory trials are being conducted by Dr. Ralph Price, University of Arizona, to refine the levels of ammonia and water used in treating cottonseed. Until these trials are completed, the recommended treatment based on Arizona trials and experiences is:

- 1 1/2 per cent ammonia
- 10 per cent water
- to remain sealed for 2 weeks
- expose to air 28 to 48 hours before feeding

Observations and Conclusions

1. Elevated moisture (17-20% range) aids ammonia in reducing aflatoxin to safe feeding levels in cottonseed sealed in polyethylene bags at atmospheric pressure and ambient temperature for 7 to 14 days.

2. Commercial equipment is available to carry out the bag process on the farm. In Arizona, 3 custom operators had processed approximately 6000 tons of cottonseed from March through June, 1979.

3. Cottonseed sealed in an ammonia atmosphere appears not to heat, mold or decay, thus the bag provides a desirable storage system.

4. No palatability problems have been encountered where bag treated seed is exposed to air 24 to 48 hours before feeding.

5. No problems have been encountered from offensive amounts of ammonia associated with the plastic bag process.

6. Custom charges for the bag process has ranged from \$15 to \$20 per ton.

7. Ammoniation increases the non-protein nitrogen content of cottonseed from 1 1/2% to 3%.

8. To date, ammoniation has NOT been approved by the F.D.A. or U.S.D.A. for aflatoxin treatment. Tests are under way for cottonseed meal treated by high pressure and for corn treated at atmospheric pressures. Results to date are encouraging. Work with radioactive tracers indicate all aflatoxin - ammonia breakdown products are eliminated with body waste. No absorption was found in the gut.

9. Clearance tests for bag treated cottonseed are being seriously considered by Arizona authorities and the agricultural industry at this time (September 1979).

10. Figure 3 illustrates the chemical structure of various aflatoxins while Figure 4 illustrates the chemical reaction in the ammoniation of aflatoxin.