Effects of Ammoniation on Aflatoxins in Rations Fed Lactating Cows

JOHN D. MCKINNEY and **GEORGE C. CAVANAGH**, Ranchers Cotton Oil,
and **J.T. BELL, A.S. HOVERSLAND, D.M. NELSON, J. PEARSON** and
R.J. SELKIRK, California State University at Fresno,
Fresno, California 93710

ABSTRACT

A multifaceted cooperative research program involving industry, government and universities was initiated to determine the effects of feeding lactating dairy cows rations containing various levels of cottonseed and cottonseed meal that had been naturally contaminated with aflatoxins. Evidence is presented that ammoniation of aflatoxin-contaminated cottonseed and cottonseed meal eliminates the aflatoxins, producing a product safe for feeding to ruminants. The aflatoxin M_1 content of milk samples of individual cows receiving rations containing (a) prime cottonseed meal, (b) aflatoxin contaminated meal, and (c) aflatoxin contaminated meal that had ammoniation treatment is reported. Data comparing results with (d) prime cottonseed, (e) aflatoxin contaminated seed, and (f) aflatoxin-contaminated seed that had ammoniation treatment are also reported. None of the milk samples from cows fed ammoniated rations contained any detectable M_1 by the modified Jacobson et al. methodology used. The sensitivity of the method in this laboratory is 0.1 μg M_1/liter of milk. Under the conditions of this study, aflatoxin M_1 levels are related to the levels of aflatoxin B_1 consumed in the diet of the cows. Conversion ratios are reported. Aflatoxin M_1 levels in the milk, relative to the time of the cows' initial ingestion of aflatoxin B_1, the persistence of M_1 in the milk after discontinuing ingestion of B_1, and disappearance of M_1 under the conditions of the analytical methodology used relative to storage time and temperatures, are reported for liquid milk and for frozen milk. Milk containing the highest level of aflatoxin M_1 was treated with rennet. An 80:20 partition of aflatoxin M_1 was observed between curd and whey, respectively.

TABLE I

Phase I

Day no.	Group C[a], μg M_1/liter milk		Group D[a], μg M_1/liter milk
	Composite	Individual	Composite
1	1.1	1.1 Cow I 0.8 Cow II 1.0 Cow III 1.2 Cow IV	0.6
3	1.7		0.8
6	1.7	1.6 Cow I 1.1 Cow II 1.2 Cow III 1.7 Cow IV	
7	Feeding of aflatoxin-contaminated ration terminated		
9	Negative[b]		Negative[b]
12	Negative[b]		Negative[b]

[a]Group C received daily CSM containing 1200 μg aflatoxin B_1. Group D received daily CSM containing 600 μg aflatoxin B_1. Group A received daily CSM (ammoniated) that contained no detectable aflatoxin but contained 1800 μg B_1 before ammoniation. Group B received daily CSM (ammoniated) that contained no detectable aflatoxin but contained 600 μg B_1 before ammoniation.

[b]In all of the milk from groups A and B and on day 9 and 12 from groups C and D, no aflatoxin M_1 was detected by analytical method used with a sensitivity of 0.1 μg/liter.

INTRODUCTION

In 1963 Allcroft and Carnaghan (1) reported that cows fed rations containing groundnut meal contaminated with aflatoxin B_1 excreted a toxic factor in their milk that had biological effects on day-old ducklings similar to those caused by aflatoxin B_1.

The nature of this toxic material in cows' milk was studied further in 1964 by de Iongh et al. (2). Extracts of lyophilized milk, obtained from cows fed rations containing contaminated peanut meal, were analyzed by thin layer chromatography (TLC). Little, if any, aflatoxin B_1 was present in the extract. A violet fluorescent substance with an Rf much lower than that of aflatoxin B_1 was observed. Duckling bioassay of this fraction of the extract indicated that toxicity of the milk was due to this fluorescent "milk toxin" substance.

The chemical nature and structure of the "milk toxins" were determined to be the monohydroxy derivatives of aflatoxin B_1 and B_2 by Holzapfel et al. (3) and also by Masri et al. (4). They were designated as aflatoxin M_1 and M_2, respectively.

In 1964 de Iongh et al. (2) reported that the toxic principle in the milk of lactating rats fed rations containing chromatographically pure aflatoxin B_1 represented a metabolic product of aflatoxin B_1 which was later termed aflatoxin M_1.

In 1966 Holzapfel et al. (3) conducted aflatoxin M_1 toxicity tests with day-old Peking ducklings and found LD_{50} values of B_1 and M_1 at 12 and 16.6 μg, respectively. The toxicity of M_1 appears to be of the same order as B_1. Aflatoxin M_2 is less toxic than aflatoxin M_1.

Masri and coworkers (5) concluded that, when high levels of spiked aflatoxins are fed, ca. 1-3% of the mycotoxin ingested is transmitted into the milk as M_1. A rapid drop of M_1 in the milk when the intake of aflatoxin is discontinued was also noted by these authors.

Both the acute and chronic toxicity (6) of aflatoxin M_1 indicate the public health concern associated with consumption of milk and milk products obtained from dairy cows ingesting feeds containing high levels of aflatoxins.

Industry and responsible government agencies have cooperated effectively to protect consumers from the recently discovered potential hazards of aflatoxin contamination in agricultural crops. The basic approach has been to exclude contaminated lots from food and feed channels. Although necessary, this has resulted in economic hardship to growers and processors of, among other crops, peanuts and oilseeds grown in areas of high aflatoxin contamination.

Earlier Cavanagh and Ensminger (7) had established the efficacy of ammoniation as a means of adding nonprotein nitrogen to cottonseed meal for ruminants through extensive experimentation and feeding trials conducted at Washington State University (8), University of California, Davis (9) and California State University, Fresno (10).

In 1966 Dollear and Gardner (11) reported on the chemical inactivation of aflatoxin in cottonseed and peanut

TABLE II

Phase II: Cows Fed Aflatoxin-Contaminated Ration (5.82 mg B_1/day)

Day no.	Cow, ID	Total milk production, liters	Concentration M_1 in milk, μg/liter	Conversion ratio, % M_1 (milk)/B_1 (ration)
1	I	10.9	Negative by procedure with sensitivity of 0.1 μg/liter	
	II	13.2		
	III	5.9		
	IV	5.3		
2	I	11.1	3.4	0.62
	II	13.5	4.0	.68
	III	6.3	2.5	.26
	IV	6.0	5.0	.51
3	I	10.9	5.3	1.04
	II	14.2	4.0	.93
	III	6.6	3.6	.38
	IV	5.6	5.0	.48
4	I	10.9	5.3	1.04
	II	15.5	4.0	1.01
	III	7.0	5.3	0.60
	IV	7.5	4.8	0.62
5	I	11.7	5.0	.96
	II	14.8	5.0	1.22
	III	6.1	5.3	0.53
	IV	6.6	5.3	0.60
6	I	11.5	5.3	1.00
	II	14.9	4.0	0.97
	III	6.6	4.0	0.43
	IV	6.6	5.1	0.56
7	I	11.6	5.0	0.94
	II	15.2	4.7	1.12
	III	7.0	5.3	0.60
	IV	6.6	5.0	0.57
8	I	12.6	5.3	1.09
	II	16.0	5.3	1.38
	III	6.2	4.7	0.45
	IV	6.9	5.3	0.63
9	I	11.6	5.0	0.94
	II	15.4	4.0	1.00
	III	6.5	4.7	0.49
	IV	6.7	5.0	0.58

meals by ammoniation, where 98-100% of the aflatoxins were eliminated as determined by TLC. Goldblatt (6), in 1968, reported that biological tests of the treated meal indicated elimination of toxicity. Gardner et al. (12) conducted large scale tests to eliminate aflatoxins by ammoniation at Ranchers Cotton Oil, utilizing equipment used to ammoniate cottonseed meal for ruminant feeding. During these tests, 2000-25-- lb lots of a meal containing 519 ppb total aflatoxin were ammoniated, and the aflatoxin content reduced to 5 ppb and nondetectable levels.

As a result of these findings, the present dairy cattle feeding trials were initiated, to determine the biological effectiveness of ammoniation as a means of inactivating aflatoxins occurring naturally in contaminated cottonseed and cottonseed meal, as well as studies of the physiological effects and metabolic fate of aflatoxins fed to dairy cattle. This paper reports the analytical aspects of the investigation. The toxicological aspects will be reported elsewhere.

EXPERIMENTAL PROCEDURES

The experimental design for this series of dairy cattle feeding trials was initiated at a meeting with FDA officials and industry representatives in Washington, D.C., October 7, 1971. The purpose of the experiment was to determine the efficacy of ammoniation in inactivating the deleterious effects of aflatoxins in cottonseed and cottonseed meal.

The California State University, Fresno, Animal Science Department conducted the feeding trials with industry providing ammoniated and unammoniated aflatoxin-contaminated cottonseed and solvent extracted cottonseed meal. The conditions for ammoniation were similar to those described by Gardner et al. (12). Ranchers Cotton Oil provided laboratory determinations of aflatoxin levels in feed ingredients and rations, and aflatoxin M_1 determinations of milk samples.

This feeding trial research program was divided into three phases. Phase I consisted of feeding ammoniated and *unammoniated* aflatoxin-contaminated solvent-extracted cottonseed meal. Twenty Holstein dairy cows were divided into four groups of four cows according to their feed consumption and milk production, with each group having an alternate cow in case any refused to eat during the feeding trial. The first 7 days of the experiment served as an orientation period during which all animals were daily fed an aflatoxin-free diet consisting of: 12 lb cottonseed meal (CSM), 9 lb mixed grain ration, 10 lb wet beet pulp (2 lb dry wt), and 10 lb alfalfa hay. One half of this ration was fed at each feding period, at intervals of 12 hr. Cows were milked during each feeding period. Two groups of cows were brought into the milking barn simultaneously and placed at opposite ends of the feed trough where board dividers were fitted to prevent the more aggressive cows from consuming the diets of the slower eating cows. At the conclusion of the feeding and milking of two groups of cows, the feed tough was washed thoroughly and the remaining two groups of cows were brought in for feeding and milking.

A portable milking machine was utilized for milking. After each milking the machine was washed thoroughly with cold, clean water. Plastic containers with a volume of ca. 300 ml were used for collection of all milk samples for analysis. Between milkings the cows were quartered in a dry lot where only water was accessible. The milk samples were collected daily, refrigerated, and delivered the following morning to Ranchers Cotton Oil laboratory for analysis. On

TABLE III

Phase II: Concentration of Aflatoxin M_1 in Milk after Withdrawal of Contaminated Ration

Time after aflatoxin withdrawal	Cow identification	Aflatoxin M_1 in milk, μg/liter
Initial	I	5.0
	II	4.0
	III	4.7
	IV	5.0
+ 24 hr	I	1.1
	II	0.7
	III	1.1
	IV	1.8
+ 48 hr	I	1.1
	II	0.4
	III	0.2
	IV	1.0
+ 72 hr	I	Trace
	II	Trace
	III	Negative[a]
	IV	Trace
+ 96 hr	I	Negative[a]
	II	Negative[a]
	III	Negative[a]
	IV	Negative[a]

[a]No aflatoxin M_1 was detected by procedure used with sensitivity of 0.1 μg/liter.

the last day of the 7 day orientation period, the total milk production from one milking was kept for lyophilization to be used in duckling feeding tests as a control. Blood samples were taken from individual cows for blood chemistry studies. Feed consumption and milk production records were kept on individual cows throughout the entire investigation. In addition, duplicate 300 ml daily milk samples from each cow were frozen and retained for future study.

At the initiation of the feeding of ammoniated and aflatoxin-contaminated meal, the total daily ration remained identical except for substitution of the experimental meals for the aflatoxin-free cottonseed meal fed during the control period. Group A: 12 lb aflatoxin-contaminated CSM (ammoniated). Aflatoxin B_1 = nondetectable by AOAC/AOCS Method 26.031-.038 which has a sensitivity in this laboratory of 1 μg/kg on aliquots of a single extracted sample. Group B: 6 lb aflatoxin-free CSM plus 6 lb aflatoxin-contaminated CSM (ammoniated). Aflatoxin B_1 = nondetectable. Group C: 12 lb aflatoxin-contaminated CSM. Aflatoxin B_1 = 220 ppb, = 1200 μg B_1/head/day, = 69.6 μg B_1/kg ration. Group D: 6 lb aflatoxin-free CSM plus 6 lb aflatoxin-contaminated CSM. Aflatoxin B_1 = 115 ppb, = 600 μg B_1/head/day, = 34.8 μg B_1/kg ration.

This feeding program was continued for a period of 14 days during which group composite milk samples, as well as milk samples from individual cows in group C, were analyzed for aflatoxin M_1 content. Blood samples were taken from individual cows at 7 day intervals. Liver biopsies were taken from two cows in each group at the termination of experimental diets. Liver biopsies were analyzed, and no aflatoxin found in the small samples submitted. At the condlusion of the 14 day feeding trials, the cows were again placed on the same uncontaminated rations used in the initial orientation period. Collection and analysis of group milk samples continued during the 10 day period following withdrawal of the experimental diets.

Phase II of the aflatoxin feeding trial consisted of feeding rations containing aflatoxin-contaminated and aflatoxin-contaminated, ammoniated cottonseed. This feeding trial resulted in the cows consuming much higher levels of aflatoxin B_1, because the cottonseed contained an arithmetic average of 2600 ppb aflatoxin B_1. A portion of this highly contaminated seed was ammoniated and contained no detectable aflatoxin B_1 by TLC or by bioassay after ammoniation.

Phase II feeding trials were conducted much the same as the initial phase I program, except that nine cows divided into two groups were used in this experiment. One group of five cows was fed rations containing the ammoniated contaminated seed, while another group of four cows was fed rations containing the aflatoxin contaminated seed.

For a control period of 4 days, the nine cows in these two groups were placed on an orientation ration consisting of: 6 lb whole prime cottonseed, 12 lb wet beet pulp (2 lb dry wt), 10 lb grain mixture, and 10 lb alfalfa hay. As in phase I, body weights and blood samples were taken prior to, during and following, the feeding of ammoniated and contaminated rations. Blood chemistry and tissue analysis will be reported when completed.

At the conclusion of the 4 day orientation period, 6 lb aflatoxin-contaminated seed replaced the 6 lb prime seed in the rations of four cows designated I, II, III and IV. Ammoniated seed replaced the 6 lb prime seed in the rations of five cows. The feeding of the experimental rations continued for a period of 9 days. Milk samples from the group receiving the ammoniated, contaminated seed were composited and analyzed as a group sample, while individual milk samples from cows on the aflatoxin-contaminated diet were analyzed each day. Additional individual milk samples were frozen each day for future study. Lyophilized milk is being tested with ducklings and with trout. This work will be reported at a later date.

The phase III feeding trial was conducted for the purpose of determining biological and toxicological effects upon the cows, when they were fed relatively high levels of both ammoniated and unammoniated cottonseed that was contaminated with high levels of aflatoxin.

For this test 18 nonlactating Holstein cows were divided into three groups of six cows. All groups were fed the same basic ration as in phase II, with the control group fed prime clean cottonseed. Another group was fed ammoniated, aflatoxin-contaminated cottonseed. A third group was fed aflatoxin-contaminated cottonseed.

This feeding trial continued for a period of 14 days. All cows were offered 8 lb cottonseed per day for the first 7 days and 10 lb per day for the second 7 days. For the aflatoxin group this resulted in each cow consuming 9.43 mg aflatoxin B_1 per day for the first 7 day period and 11.79 mg aflatoxin per day for the second 7 day period, for a total consumption of 148.54 mg aflatoxin B_1 for the

TABLE IV

Aflatoxin M_1 Disappearance from Raw and Frozen Milk Samples

A. Liquid Raw Milk, 32 F

Day no.	Aflatoxin M_1 μg/liter Sample 1	Sample 2
Fresh	1.3	5.3
2	1.3	5.2
4	0.8	4.8
6	0.3	4.0
8	0.2	0.6
12	Negative[a]	0.6

B. Frozen Raw Milk, 0 F

Day no.	Aflatoxin M_1, μg/kg Sample 1	Sample 2
Fresh	5.0	5.3
10	5.0	Sample lost
30	4.8	4.0
50	2.9	2.2
120	Insufficient sample for analysis	0.7

[a]No aflatoxin M_1 detected by procedure used with sensitivity of 0.1 μg/liter.

total period of the feeding trial. Individual body weights were taken before and after the feeding period.

At the termination of this feeding trial, cows were sacrificed for toxicological studies now in progress. Only the results of chemical analysis of tissues for aflatoxins will be presented in this report.

Analysis of milk samples was by the method of Jacobson et al. (13) with modifications developed in our laboratory (14), which produced sufficiently clean extracts to allow determination of aflatoxin M_1 in raw milk at levels of 0.1 ppb with confidence. Recovery of aflatoxin M_1 in spiked milk samples gave an average recovery of 91%. TLC methods of Stubblefield et al. (15) were used. The very sensitive chemical confirmatory test of Stack et al. (16) was used to confirm the presence of aflatoxin M_1. Further confirmation of the presence of aflatoxin M_1 in milk samples was made by W.F. Haddon, USDA Western Regional Research Lab., who provided mass spectrograph (MS) analysis (17) for positive identification.

RESULTS AND DISCUSSION

To confirm that aflatoxins are chemically altered by ammoniation and are not reactivated by the acid in animals stomachs, the following test was performed: 500 g dehulled ammoniated, contaminated cottonseed, initially containing 2700 μg/kg total aflatoxins, was analyzed by the official procedure for aflatoxins in cottonseed and none was detected. Another sample of the ammoniated, contaminated cottonseed described above was slurried with 2 liters 3 N HCl in a one gal Waring Blendor. Aliquots of this slurry were analyzed for aflatoxins at intervals of 3, 6 and 24 hr and found to be negative. 100 g aliquot of this slurry was extracted, cleaned by AOCS procedure and this cleaned-up extract spotted on preparatory TLC plates. The band at the Rf of aflatoxin B_1 was removed from the plate and submitted to W.F. Haddon at Western Regional Research Lab. for confirmatory MS analysis (17). No aflatoxin was detected.

Table I shows the aflatoxin M_1 content of milk samples from the phase I feeding trials. Composite samples of milk from all groups of cows were analyzed, as well as individual cows from group C which were fed the highest levels of aflatoxin-contaminated cottonseed meal.

TABLE V

Aflatoxin M_1 Distribution in Rennet Precipitation of Naturally Contaminated Milk Containing 4.4 μg/liter Aflatoxin M_1

Curd,[a] μg/kg	Whey, μg/liter
A = 9.2	A = 0.6
B = 9.3	B = 0.5
C = 9.2	C = 0.6

[a]Approximate 20:80 separation of milk into curd and whey.

Milk samples from groups C and D, fed rations containing aflatoxin B_1, indicated that within 24 hr after initial ingestion of aflatoxin B_1, aflatoxin M_1 appears in the milk, and that within a period of 72 hr the level of aflatoxin M_1 had reached the maximum level attained during the phase I feeding trial. Aflatoxin M_1 levels in the milk were proportional to the level of ingested aflatoxin B_1 with the cows receiving ca. 1200 μg B_1 per day, producing milk containing approximately twice the aflatoxin M_1 content of cows receiving ca. 600 μg B_1 per day. Milk samples of individual cows varied from one another in aflatoxin M_1 content when fed equal levels of aflatoxin B_1 in their rations. Within 24 hr after contaminated rations were withdrawn, no detectable aflatoxin M_1 was present in the milk.

Table II shows data obtained from the phase II feeding trial. This consisted of feeding rations containing ammoniated and unammoniated aflatoxin-contaminated cottonseed for a period of 9 days. All group milk samples from cows fed rations containing aflatoxin-contaminated seed that had been ammoniated contained no detectable aflatoxin M_1. This table relates to those cows receiving rations containing contaminated seed that resulted in their ingesting 5.82 mg per day of naturally occurring aflatoxin B_1. Palatability presented no problems with either regular or ammoniated seed. The relatively short period of the feeding trial probably accounts for the relatively constant total daily milk production of individual cows. Just as in phase I feeding trials, aflatoxin M_1 is detectable in milk within 24 hr after the aflatoxin-containing diet has been ingested. The concentration of aflatoxin M_1 in the milk attains a maximum ca. 3 days after the cows have been on the aflatoxin B_1-contaminated ration. The concentration of aflatoxin M_1 in the milk showed no correlation to the milk yields.

Table II also shows the percentages of ingested aflatoxin B_1 converted to M_1 in the milk of individual cows. The cows with the highest milk production appeared to convert higher percentages of aflatoxin B_1 to M_1. These conversion ratios after the 72 hr period, during which maximum conversion of B_1 to M_1 is reached, vary under the conditions of this experiment from 0.43% to 1.38%. These values compare favorably with those of Masri et al. (5), who in 1968 conducted a long term feeding trial and obtained ratios averaging 1-2%. It would appear that the amounts of aflatoxin B_1 in the ration converted to M_1 in the milk are very small.

Table III relates to disappearance of aflatoxin M_1 from the milk after the withdrawal of aflatoxin-contaminated rations of phase II, where the rations contained higher levels of aflatoxin B_1 than were in rations of phase I. Within 72 hr after withdrawal of aflatoxin B_1, milk was essentially free of M_1. This indicates that milk produced by cows fed aflatoxin-contaminated feed would be essentially free of aflatoxin M_1 within 3-4 days following withdrawal of the contaminated feed.

Concern with the stability of aflatoxin M_1 in raw milk led to an investigation of the rate of disappearance of aflatoxin M_1 in samples of raw milk in both liquid and frozen states. Table IV shows this disappearance with respect to time. Samples were stored in a refrigerator at

TABLE VI

Tissue Analysis for Aflatoxin[a] from Cows Fed Aflatoxin-Contaminated Cottonseed

Sample no.	Aflatoxin, μg/kg		
	B_1	B_2	M_1
Liver			
49	0.1[b]	>0.025	>0.1
53	0	0	0.15
57	>0.1[b]	0.025	0.1
61	>0.1	0.025	0.1
65	0	0	0
69	0	0	Trace
Kidney			
54	0	0	0.3[c]
58	Trace	0	0.3[c]
62	<0.005	0	0.3[c]
66	0	0	<0.05
70	0	0	0.15[c]
Round			
51	No aflatoxin detected in samples from round		
55			
59			
63			
67			
71			
Heart			
52	0	0	<0.05
56	0	0	<0.05
60	0	0	<0.05
64	0	0	<0.05
68	0	0	0
72	0	0	0

[a]No aflatoxins detected in tissues of cattle fed regular or ammoniated contaminated cottonseed.

[b]Confirmed by preparation of water adduct derivative.

[c]Traces of M_2 also observed in these samples.

32-34 F and aliquots periodically withdrawn for analysis.

Aflatoxin M_1 in liquid raw milk samples disappears very rapidly in storage, with the result that in samples with lower aflatoxin M_1 levels ca. 40% of initial aflatoxin M_1 is not detectable after a period of 4 days and ca. 80% after a period of 6 days when stored at ca. 32 F. Frozen raw milk samples indicate a slower, but nevertheless persistent, rate of disappearance of aflatoxin M_1. Analyses of frozen curd and whey samples obtained from a rennet precipitation of aflatoxin-contaminated milk indicate little or no degradation of aflatoxin M_1 content after a period of 4 months. Possibly the relatively rapid apparent degradation of aflatoxin M_1 results from some enzymatic system naturally present in raw fresh milk, which is inactivated in the rennet precipitation processing or lyophilization of raw milk, or possibly the aflatoxin M_1 is bound to protein and is not extractable by the solvent system used in the method of analysis. Aflatoxin M_1 analysis of raw milk samples should therefore be carried out immediately after collection. If this is not possible, then samples should be quick-frozen and analyzed as soon as possible. Any extended delay could result in unreliable results.

Table V tabulates the results of the partitioning behavior of aflatoxin M_1 in a rennet precipitation of aflatoxin M_1-contaminated milk into curds and whey. The aflatoxin M_1 content of the initial raw milk sample was 4.4 μg/liter. After rennet precipitation, the curd was pressed to removed entrained whey, but was not washed. This resulted in ca. 20:80 separation by weight of milk into curd and whey fractions. Analyses of these two fractions indicate that ca. 80% of the aflatoxin M_1 was detected in the curd fraction and ca. 20% in the whey fraction.

Under the conditions of this experiment, the partitioning behavior of aflatoxin M_1 between curd and whey reflects an affinity for the casein fraction. This partitioning behavior has been noted previously by other investigators (Allcroft et al. [18] and Grant and Carlson [19]). Observations by others (20) indicate that in a rennet precipitation of aflatoxin-contaminated milk virtually all aflatoxin M_1 should partition in the casein fraction. These findings could be reason for concern that considerable levels of aflatoxin contamination could exist in cheese prepared from milk from cows fed aflatoxin-contaminated rations.

Table VI is a tabulation of the results of tissue analysis for aflatoxins of sacrificed cows from phase III feeding trials.

These results show that there is no evidence of any aflatoxins in any tissue of cows fed ammoniated, aflatoxin-contaminated cottonseed. They also show that there is no evidence of aflatoxin accumulation in the muscle tissue examined from cows fed aflatoxin-contaminated rations.

Of interest is the relative retention of aflatoxin in liver and kidney tissue. Liver tissue contains a higher proportion of unaltered aflatoxin B_1, whereas the M_1 metabolite is present in larger relative amounts in kidney tissue. Low levels of M_1 were detected in the heart tissue. The metabolic fate of ingested aflatoxins is a matter of practical importance in determining the extent to which edible animal tissue becomes contaminated with aflatoxin or toxic derivatives.

All of the chemical and biological data accumulated to date from this aflatoxin feeding trial has demonstrated that ammoniation is an effective and practical method for the elimination of aflatoxin in contaminated cottonseed and cottonseed meal. Research on methods of prevention of aflatoxin contamination in agricultural commodities that will eliminate the need for detoxification methods is of primary importance, but in certain geographic areas field contamination renders large amounts of cottonseed toxic and unusable. Ammoniation presents a technically feasible method for the elimination of aflatoxin that will allow its utilization in the rations of lactating cows. Aflatoxin-contaminated cottonseed or meal, properly ammoniated and

subsequently acidulated with 3 N HCl, does not result in reactivation of aflatoxins as determined by the usual solvent extraction followed by TLC or MS analysis. Experiments are underway that should show the practicality of ammoniation for the elimination of aflatoxin.

ACKNOWLEDGMENTS

Producers Cotton Oil Co., National Cottonseed Products Association and Ranchers Cotton Oil provided support for the cattle feeding trials. Imperial Western Products, Inc., and ARS, USDA provided support for toxicological studies. Tissue analysis in Table VI was done by S. Nesheim and N. Brown, Div. of Chemistry and Physics, FDA, Washington, D.C. Protocol-experimental Design: H. Fischbach, Office of Science, FDA, Washington, D.C., and A.D. Campbell and J. Rodricks, Div. of Chemistry and Physics, FDA, Washington, D.C.

REFERENCES

1. Allcroft, R., and R.B.A. Carnaghan, Vet. Rec. 75:250 (1963).
2. de Iongh, H., R.O. Vles and J.G. Van Pelt, Nature 202:466 (1964).
3. Holzapfel, C.W., P.S. Steyn and I.F.H. Purchase, Tetrahedron Lett. 25:2799 (1966).
4. Masri, M.S., R.F. Lundin, J.R. Page and V.C. Garcia, Nature 215:753 (1967).
5. Masri, M.S., V.C. Garcia and J.R. Page, Vet. Rec. 81:146 (1969).
6. Goldblatt, L.A., Econ. Bot. 22:51 (1968).
7. Cavanagh, G.C., and M.E. Ensminger, Livestock Bus. 1:1 (1968).
8. Heinemann, W.W., Bull. 666, Washington State University, Prosser Experiment Station, 1965.
9. Hinman, N.H., G.P. Lofgreen and W.N. Garrett, Calif. Agri. 20(8):13 (1966).
10. Pearson, J., M.S. thesis, California State University, Fresno, 1970.
11. Dollear, F.G., and H.K. Gardner Jr., Proc. Fourth Nat. Peanut Res. Conf., Tifton, Ga. July 1972, 1966.
12. Gardner, H.K., Jr., S.P. Kiltun, F.G. Dollear and E.T. Rayner, JAOCS 48:70 (1971).
13. Jacobson, W.C., W.C. Harmeyer and H.G. Wiseman, J. Dairy Sci. 54:21 (1971).
14. McKinney, J.D., JAOCS 49:444 (1972).
15. Stubblefield, R.O., O.L. Shotwell and G.M. Shannon, J. Ass. Office. Anaol. Chem. 55:762 (1972).
16. Stack, M.F., A.E. Pohland, Dantzman, and S. Nesheim, Ibid. 55:313 (1972).
17. Haddon, W.F., M. Wiley and A.C. Waiss, Jr., Anal. Chem. 43:268 (1971).
18. Allcroft, R., and R.B.A. Carnaghan, Vet. Rec. 75:259 (1963).
19. Grant, O.W., and F.W. Carlson, Bull. Envir. Cont. and Toxicol. 6:521 (1971).
20. Kraybill, H.F., and R.E. Shapiro, "Aflatoxins:Scientific Background, Control and Implications," Edited by L.A. Goldblatt, Academic Press, New York, 1969, p. 412.

[Received October 9, 1972]