Transmission of Aflatoxin B_1 into the Tissues of Growing Pigs

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Aflatoxin transmission into tissues of pigs after feeding of levels of aflatoxin that did not produce anorexia, impairment of feed efficiency, or pathological effects has not been reported. The purpose of this study was to determine if aflatoxin residues could be measured in tissues of swine receiving aflatoxin in the diet at levels approximating the range of field contamination.

The transmission of aflatoxins into foods of animal origin was reviewed by PURCHASE (1972). Later MABEE and CHIPLEY (1973) administered low levels of ^{14}C-labeled aflatoxins to broiler chickens by crop intubation. The radioactivity detected in the liver, heart, gizzard, breast meat, and leg meat accounted for 9.36% of the total ^{14}C administered. These workers prepared a pooled sample of lyophilized radioactive excreta, blood, organs, and tissue. According to their analysis, 81% of the radioactivity observed in this combined sample was confined to the sodium acetate buffer solution. The extract was treated with β-glucuronidase and then extracted with chloroform; 31.5% of the radioactivity was present in the chloroform extract. Thin-layer chromatography (TLC) of the chloroform extract showed that the activity was due to a compound assumed to be a conjugate of aflatoxin M_1.

KROGH et al. (1973) fed diets containing 300 or 500 μg of aflatoxins B_1 + B_2/kg feed to pigs for 120 to 231 days. During the growth period from 20 to 90 kg, the pigs on the aflatoxin-contaminated diets had impaired weight gain and feed conversion. The pigs were slaughtered 4 hr after the last feeding, and tissue samples were analyzed. Various amounts of aflatoxins B_1, B_2, and M were found in the liver and kidney, and trace amounts (<1 ppb) of these compounds were found in the heart, muscle, and adipose tissue of the pigs on the contaminated diet. The liver of one of the 4 control pigs showed traces of aflatoxin M.

MURTHY et al. (1975) reported that the response of swine to aflatoxin depends on whether the aflatoxin-contaminated protein source is fed separately or is incorporated in the total diet ration. In their study, pigs fed the aflatoxin source separately developed toxic symptoms and aflatoxins B_1, B_2, and M were found in tissues. The pigs fed the mixed diet did not develop toxic

[1] Retired.

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symptoms; no aflatoxin residues were found in the tissues of the one pig examined.

Materials and Methods

Eight female and 8 male feeder pigs, 2 females and 2 males from each of 4 different litters, were selected to form 4 balanced groups on the basis of sex and weight. The initial body weight ranged from 54.2 to 71.6 kg. The pigs were housed in individual pens and were fed aflatoxin-contaminated diets. The amount of feed offered to the pigs was calculated by the following equation (ARMBRECHT et al. 1971): $\underline{Y} = 1.0403 + 0.02925\underline{X}$, where $\underline{Y}$ = kg ration and $\underline{X}$ = body weight. All the diet offered was consumed. The pigs were weighed weekly and the diet offered was adjusted accordingly.

The composition of the diet is given in Table 1. To avoid the variability of aflatoxin contamination due to scattered pockets of mold in large masses of stored corn (ARMBRECHT et al. 1975), the corn component of the diet was taken from an 18,000 kg lot of corn that has been picker-shelled and heat-dried (supplied by the Iowa State University). The ground corn contained 0.1 µg each of aflatoxins B_1 and G_1/kg and 92% dry matter; these amounts of aflatoxins are too low to have any effect on the control group. This corn was used for both the experimental and the control diets.

TABLE 1

Composition of basal diet

Ingredients	%
Ground corn yellow	75.0
Soybean meal	8.75
Linseed meal	5.00
Afalfa meal	5.00
Fish meal	2.5
Tankage	2.5
Mineral mix[a]	1.25
	100.00

[a]Comprised of iodized salt 33%, steamed bone meal 33%, ground limestone 31.3%, ferrous sulfate 2%, zinc oxide 0.4%, manganous sulfate 0.2%, copper sulfate 0.1%.

The ground corn was examined for aflatoxins by the procedure described by JACOBSON et al. (1971). The spot presumed to be aflatoxin was scraped from the TLC plate and aflatoxin was eluted from the scrapings with chloroform-ethanol (99+1). The eluant was filtered and the identity of the aflatoxin in the ground corn was confirmed by the derivative technique of WISEMAN et al. (1967b): 75 g of acid-washed Celite 545 was blended with 750 ml of hexane in a 1 liter blender; 7.5 ml of concentrated phosphoric acid was added to the mixture during blending. The mixture was blended for 2 min more and then poured in a Mason jar for storage. This mixture was added to a 10 mm id x 300 mm long chromatographic tube with a stopcock and a S 24/40 joint to give a packed column 80 mm high. The column was packed by applying nitrogen pressure (500 mm of mercury) and releasing the pressure when the hexane reached the top of the column. The hexane was then washed off the column with 10 ml of chloroform-ethanol (99+1). The sample was applied to the column, and the derivatized aflatoxin was eluted with 100 ml of chloroform-ethanol (99+1) and collected in a 250 ml flask. The eluate was evaporated, the residue was transferred to a 10 ml centrifuge tube with three 2 ml rinses of chloroform, and the chloroform was evaporated to dryness. Fifty µl of chloroform was added to dissolve the residue and the total volume was spotted on a kieselguhr-formamide-water TLC plate prepared as described by ADYE and MATELES (1964) as modified by JACOBSON et al. (1971). Standard aflatoxin and derivatized standard aflatoxin were also spotted on the TLC plate. The solvent systems and the developing techniques were the same as described by JACOBSON et al. (1971). The reaction product of standard aflatoxin B_1 was examined by mass and nuclear magnetic resonance spectroscopy and was shown to be the ethanol adduct.

Pure aflatoxin B_1, prepared as described by JACOBSON et al. (1971), was added to the basal diet at levels of 100, 200, and 400 µg/kg feed. The control and experimental diets were fed for a 4-week period. One urine passage was collected from each of the pigs on the 400 µg/kg diet during the third week. Since this experiment was conducted in July and August, these pigs were placed in collection crates early in the morning to minimize heat stress and the time required to collect the samples. At the end of 4 weeks, the pigs on the aflatoxin-contaminated diets were slaughtered early in the morning to keep the time between the last feeding and slaughter at a minimum (approximately 12 hr) and blood, muscle, liver, and kidney samples were taken on the same day.

The blood, urine, and tissue samples were analyzed by the method of JACOBSON et al. (1971) with the following modifications: 4 ml of ethanol was added to the 400 ml of chloroform extract and the pigments were removed as described by WISEMAN et al. (1967a). The chloroform extracts (400 ml) were stored at 3°C until analysis.

Results and Discussion

The control group gained an average of 15.1 kg during the 4-week feeding period. The average weight gains of the pigs on diets containing 100, 200, and 400 µg of aflatoxin B_1/kg feed were 13.6, 16.4, and 11.5 kg, respectively.

The 3-week urine samples from pigs on the 400 µg/kg diet showed an average of 0.56 µg aflatoxin B_1/kg body weight and 3.68 µg M_1/kg body weight. The individual values were 1.17, 0.44, 0.43, and 0.22 µg/kg for B_1 and 1.80, 2.72, 2.04, and 8.16 µg/kg for M_1. The presence of aflatoxin in the urine probably means that the tissue contained aflatoxins at some undetermined level; this is supported by the levels of aflatoxins found after the 4-week feeding period.

The aflatoxin residues found in this study are shown in Table 2; appreciable amounts of B_1 were found in samples from pigs fed the B_1-contaminated diet and all except two samples (both muscle samples) contained measurable amounts of M_1. These data agree with those reported by KROGH et al. (1973) and MURTHY et al. (1975) for the transmission of B_1 into liver and kidney tissues. From this study it seems that aflatoxin B_1 residues may be found in measurable amounts in tissues of swine fed 100, 200, and 400 µg of aflatoxin B_1/kg. Gross examination of liver tissues revealed no evidence of pathological conditions.

The values for the aflatoxin content of the diets and the residues were transformed into logarithms. A simple regression analysis was performed and the correlation coefficients were calculated. The data (Table 3) show that there is a linear relationship between the logarithms of aflatoxin B_1 intake and amounts of residues in the tissues. The data also indicate that liver is the best tissue to use for monitoring and demonstrating the transmission of aflatoxins into the tissues.

Only 2 livers and 3 kidneys from control animals were analyzed because the diet the control pigs were receiving contained <1 µg of total aflatoxins/kg and because no measurable amounts of aflatoxins were found. Aflatoxin was detected in these organs, but the amounts could not be quantitated by visual comparison with standards.

TABLE 2

Aflatoxin B_1 and M_1 content (micrograms per kilogram) of swine tissues on a wet matter basis

Aflatoxin level, µg/kg of ration	Sex of pig	Liver		Muscle		Blood		Kidney	
		B_1	M_1	B_1	M_1	B_1	M_1	B_1	M_1
400	F	0.7	1.02	0.56	0	0.32	0.11	10.0	0.41
400	F	1.62	1.34	2.22	0.35	0.48	0.15	5.62	0.18
400	M	2.66	1.36	0.36	0.17	0.48	0.23	0.63	0.68
400	M	1.04	2.00	1.04	0.32	3.33	0.06	1.51	0.23
Av.		1.51	1.43	1.04	0.21	1.15	0.14	4.44	0.38
200	F	0.26	0.41	0.19	0.04	0.39	0.07	0.23	0.29
200	F	0.18	0.32	0.43	0.08	0.21	0.18	1.50	1.08
200	M	0.75	1.50	0.69	0.09	0.26	0.11	0.34	0.34
200	M	0.74	0.11	0.51	0.06	0.47	0.11	0.73	1.29
Av.		0.48	0.58	0.46	0.07	0.33	0.12	0.70	0.75
100	M	0.18	0.11	0.20	0.04	0.03	0.06	0.37	0.09
100	F	0.25	0.16	0.23	0	0.30	0.11	0.35	0.20
100	M	0.25	0.23	0.20	0.04	0.17	0.04	0.10	0.23
100	F	0.25	0.05	0.13	0.03	0.17	0.04	0.10	0.20
Av.		0.23	0.14	0.19	0.03	0.17	0.06	0.23	0.18
Control	F	- [a]	-	-	-	-	-	-	-
Control	M	<0.12	<0.03	-	-	-	-	<0.12	<0.05
Control	F	-	-	-	-	-	-	<0.12	<0.05
Control	M	<0.12	<0.03	-	-	-	-	<0.12	<0.07

[a] - indicates that the sample was not analyzed.

Further work should be done to determine the withdrawal period needed for pigs on aflatoxin-contaminated diets. The stability and the fate of the residual aflatoxin in meat stored for short periods should also be studied.

TABLE 3

Correlation coefficients of logarithms of aflatoxin B_1 intake and aflatoxin B_1 and M_1 residues in the tissues

Tissue	B_1	M_1
Liver	0.727**	0.807**
Muscle	0.624*	0.659*
Blood	0.474	0.551
Kidney	0.592*	0.296

*Significant at the 5% level.
**Significant at the 1% level.

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