AFLATOXIN RESIDUES IN BACON PIGS

P. KROGH[a], B. HALD[a], E. HASSELAGER[b], A. MADSEN[c], H. P. MORTENSEN[c], A. E. LARSEN[c] and A. D. CAMPBELL[d]

(a) *Institute of Hygiene and Microbiology*
and
(b) *Department of Pharmacology and Toxicology of the Royal Veterinary and Agricultural University, Copenhagen, Denmark*
(c) *Department for Experiments with Pigs, National Institute of Animal Science, Copenhagen, Denmark*
(d) *Division of Food Chemistry and Physics, Food and Drug Administration, Washington, DC, USA*

ABSTRACT

Bacon pigs were fed diets containing 300 and 500 ppb aflatoxin $B_1 + B_2$ during a long period (120–231 days). When organs and tissues were examined for residues, sizable amounts of aflatoxin (max. 54 ppb) were detected. Since macroscopic alterations were slight in livers and absent in other organs and tissues, most livers and all other organs and carcasses could have passed meat inspection and been distributed for human consumption. This calls for careful control of animal feed, in order to avoid the use of heavily contaminated batches.

Information on aflatoxin residues in food of animal origin has so far been restricted to the excretion of aflatoxins, including aflatoxin M, in the milk of lactating animals. Purchase[1] has recently reviewed this aspect extensively. Although aflatoxin has been found in liver and kidney of small laboratory animals after a single dose of aflatoxin, attempts to detect aflatoxin in organs and carcasses of farm animals have mainly been unsuccessful. No aflatoxin residue was found in a pig that died of aflatoxicoses[2], or in pigs fed various levels of aflatoxin up to 810 ppb[3]. In cattle the same negative results were obtained[2,3] although the American team[3] reported that traces of aflatoxin B_1 and M_1 were present in the blood of steers receiving a diet containing 1 ppm aflatoxin. However, 'aflatoxin or aflatoxin metabolites' were found in muscle tissue of chickens fed aflatoxin[4].

The problem of the transmission of mycotoxins through farm animals to the human food channel is very important, and may be especially pronounced for countries having extensive export of food of animal origin. Most of these countries, at least in Europe, are dependent upon an equally extensive import of feed, mostly from tropical and subtropical areas, and consequently this imported feed may be contaminated with mycotoxins, such as aflatoxin.

This again involves control for aflatoxin in the imported feed. It seems that the exposure to aflatoxin in the Northern European countries is strictly associated with the importation of aflatoxin, as this particular mycotoxin is not produced in the colder climatic areas of Europe.

In order to elucidate the possibility of residues in Danish pigs fed aflatoxin an investigation was carried out. Pigs were continuously fed a ration containing aflatoxin-contaminated peanut meal (naturally contaminated with 2500 ppb of aflatoxin B_1 and 500 ppb of B_2) and studies were performed in order to detect aflatoxin residues in organs and carcasses.

Ten blocks of pigs of Danish Landrace, each comprising three castrated males and three females, were purchased at about eight weeks of age. Within each block the six animals were distributed in three experimental groups consisting of two pigs, one of each sex, thus resulting in 20 animals on each diet. During the pre-experimental period all pigs were fed a ration of barley and protein concentrates. From 20 kg up to slaughter at 90 kg live weight, the pigs were kept in individual pens with concrete floors and straw as bedding, and maintained at a temperature of 17°C. Body weight was recorded at least every second week. Feed was weighed out daily. The pigs were fed twice a day, with daily feed allowance according to *Table 1*. The composition of the diets is shown in *Table 2*. The three diets were analysed and selected results are presented in *Table 3*.

Table 1. Daily feed allowance

Body weight (kg)	20	30	40	50	70	90
Feed mixture (kg)						
Diet 1	0.9	1.2	1.6	2.0	2.6	3.2
Diet 2	0.9	1.2	1.6	1.9	2.4	2.8
Diet 3	0.8	1.1	1.4	1.8	2.2	2.5

Table 2. Composition of diets

Ingredients	Diet 1 (%)	Diet 2 (%)	Diet 3 (%)
Barley	77.6	83.2	81.5
Soybean meal	20.0	—	—
Peanut meal	—	10.6	16.0
Meat and bone meal	—	5.3	—
Sodium chloride	0.4	0.4	0.4
Calcium carbonate	0.8	0.4	0.9
Dicalcium phosphate	1.1	—	1.1
Vitamin-trace mineral mixture*	0.1	0.1	0.1
Total	100.0	100.0	100.0

*Each gramme contained: in I.U., vitamin A 3000; vitamin D_3 600: in mg, riboflavin 5; *d*-pantothenic acid 15; vitamin B_{12} 0.02; alphatocopherol acetate 20; zinc oxide 100; copper sulphate 125; iron sulphate 125; manganese sulphate 125; cobalt sulphate 5; potassium iodide 1.

Table 3. Selected results of feed analysis

Content	Diet 1	Diet 2	Diet 3
Lysine, (g per 16 g N)	5.5	4.0	3.7
Methionine, (g per 16 g N)	1.6	1.5	1.4
Aflatoxin (B_1 + B_2), (ppb)	0	300	500

During the experiment, the pigs fed diets 2 and 3 often showed loss of appetite. This was especially pronounced for 4 pigs, two of which died after 75 (diet 2) and 137 (diet 3) days at live weights of 41 and 24 kg, respectively. After 156 and 170 days, two pigs on diets two and three at live weights of 53 and 52 kg were sacrificed for pathological examination.

During the period from 20 to 90 kg the pigs on diets two and three were much inferior to the pigs on diet one with respect to growth rate and feed conversion.

The pigs were fed for the last time at 7 a.m., as is the custom in Danish husbandry, and slaughtered at 11 a.m. the same day, at the same dressed weight (64 kg).

The pigs on diet one contained more meat and less fat than the pigs on diets two and three.

Whether the difference in carcass quality is due to the aflatoxin-contaminated feed cannot be evaluated on the basis of the present investigations.

At slaughter the pigs were inspected, and pieces of liver, kidney and heart were fixed in Lillie's neutral buffered formalin. Fixed tissues were processed for paraffin section and stained with iron hematoxylin van Gieson. At necropsy the livers of the pigs Nos. 1–12 and 19–26 (*Tables 4* and *5*) were slightly altered in the form of a slight cirrhosis, which did not cause rejection during meat inspection. The livers of the pigs Nos. 13–18 and 27–36 (*Tables 4* and *5*) had a yellow discolouration and rather pronounced cirrhosis; these livers would be rejected during meat inspection. All other organs, including the kidneys and all carcasses were free of any macroscopical alteration, and would therefore pass the meat inspection.

At histological examination, the livers showed a varied pathological picture, including cirrhosis, karyomegaly, atypical nuclei and proliferation of the bile ductules. The kidneys of some of the pigs (*Tables 4* and *5*) displayed microscopically a tubular degeneration consisting of very enlarged nuclei followed by cell desquamation.

Four of the twenty pigs (control) fed diet 1 were selected for pathological examination. No alteration was found in organs and tissues.

To detect aflatoxin, tissue samples of liver, kidney, heart, muscle and adipose tissue were extracted as follows. Samples of 100 g each of liver, kidney, heart and muscle were homogenized with 500 ml of 70 per cent aqueous acetone, submerged in ice water to counteract the heat development, and extracted as described by Pons and Goldblatt[4a], modified by a further clean-up step on a silica gel column.

Samples of 50 g of adipose tissue were slurried with sand to a homogeneous mass, 250 ml of chloroform and Celite 545 were added, and the mixture was

Table 4. Residue and liver-kidney damage in swine fed 300 ppb aflatoxin*

Swine No.	Feeding period (days)	Aflatoxin residue (ppb) Liver B_1	Liver B_2	Liver M	Kidney B_1	Kidney B_2	Kidney M	Heart B_1	Heart B_2	Heart M	Muscle B_1	Muscle B_2	Muscle M	Adipose tissue B_1	Adipose tissue B_2	Adipose tissue M	Microscopic alteration Liver	Microscopic alteration Kidney
Liver not rejected at meat inspection																		
1	164	10	tr	0	10	tr	0	0	0	0	0	0	0	tr	0	0	+	no
2	140	10	10	0	10	0	0	0	0	0	0	0	0	tr	0	0	+	+
3	180	10	10	0	10	10	0	0	0	0	tr	0	0	0	0	0	+	+
4	120	10	0	0	10	0	0	0	0	0	0	0	0	0	0	0	no	+
5	120	10	0	0	tr	0	0	0	0	0	—	—	—	0	0	0	no	no
6	120	10	10	3	10	0	3	0	0	0	0	0	0	0	0	0	no	+
7	144	0	0	0	0	0	0	0	0	0	0	0	0	tr	0	0	no	no
8	141	tr	0	tr	tr	tr	0	0	0	0	0	0	0	0	0	0	+	+
9	164	tr	tr	tr	tr	tr	tr	0	0	0	0	0	0	tr	0	0	+	+
10	120	0	0	0	0	tr	0	0	0	0	0	0	0	0	0	0	+	no
11	120	tr	0	0	—	—	—	—	—	—	0	0	0	tr	0	0	no	no
12	133	tr	0	0	—	—	—	—	—	—	0	0	0	0	0	0	no	no
Liver rejected at meat inspection																		
13	120	10	0	0	0	0	0	0	0	0	tr	0	0	0	0	0	+	+
14	141	92	45	0	10	0	0	tr	0	0	0	0	0	tr	0	0	+	+
15	186	10	0	0	10	0	0	0	0	0	0	0	0	0	0	0	+	+
16	141	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	no	no
17	150	tr	tr	0	tr	tr	0	0	0	0	0	0	0	0	0	0	+	+
18	127	0	0	0	0	0	0	0	0	0	0	0	0	tr	0	0	+	no

*tr = trace (less than 1 ppb)

Table 5. Residue and liver-kidney damage in swine fed 500 ppb aflatoxin*

Swine No.	Feeding period (days)	Aflatoxin residue (ppb) Liver B_1	Liver B_2	Liver M	Kidney B_1	Kidney B_2	Kidney M	Heart B_1	Heart B_2	Heart M	Muscle B_1	Muscle B_2	Muscle M	Adipose tissue B_1	Adipose tissue B_2	Adipose tissue M	Microscopic alteration Liver	Microscopic alteration Kidney
Liver not rejected at meat inspection																		
19	186	11	10	tr	11	10	tr	tr	tr	0	tr	0	0	0	0	0	+	+
20	135	51	3	3	50	3	3	0	0	0	tr	0	0	tr	0	0	+	+
21	141	10	10	0	10	10	0	tr	0	0	0	0	0	0	0	0	+	no
22	141	35	15	0	0	10	0	0	0	0	0	0	0	tr	0	0	+	+
23	157	10	0	0	tr	0	0	0	0	0	0	0	0	tr	0	0	+	no
24	141	10	0	0	10	10	tr	0	0	0	tr	0	0	0	0	0	no	no
25	134	10	0	0	10	0	6	0	0	0	0	0	0	0	0	0	no	no
26	196	0	0	0	0	0	0	0	0	0	0	0	0	tr	0	0	+	+
Liver rejected at meat inspection																		
27	216	2	tr	0	2	tr	0	tr	0	0	0	0	0	tr	0	0	+	+
28	157	51	0	0	—	—	—	—	—	—	tr	0	0	tr	0	0	+	no
29	150	11	tr	tr	10	tr	tr	tr	tr	tr	0	0	0	tr	0	0	+	+
30	150	10	10	0	10	0	0	0	0	0	tr	tr	0	tr	0	0	+	+
31	159	11	10	0	tr	0	0	0	0	0	tr	tr	0	0	0	0	+	+
32	138	0	0	0	0	0	0	0	0	0	tr	0	0	tr	0	0	+	no
33	231	tr	tr	0	0	0	0	0	0	0	tr	0	0	0	0	0	+	+
34	220	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	no
35	120	tr	tr	0	tr	0	0	0	0	0	tr	0	0	0	0	0	+	+
36	141	0	0	0	0	0	0	0	0	0	tr	0	0	tr	0	0	+	+

*tr = trace (less than 1 ppb)

shaken and filtered. After evaporation to approximately 75 ml, the concentrate was placed on a silica gel column and washed with hexane and ethyl ether.

The toxins were eluted from the column with a methanol–chloroform mixture (3:97), and after evaporation to dryness quantification by t.l.c. on silica gel according to the IUPAC technique[5] was carried out. Four solvents were used as mobile phases: chloroform–acetone (9:1), chloroform–methanol (97:3), benzene–ethanol–water (46:35:19) and ethyl acetate–propanol–water (10:2:1). Quantification was achieved by visual comparison, using qualitative standards of $B_1 + B_2 + G_1 + G_2$, and M_1, as well as a quantitative standard of $B_1 + G_1$.

A preliminary investigation of recovery of aflatoxin B_1 added to homogenized liver tissue showed that 100 per cent recovery was obtained at the 1 ppb level, whereas the recovery at the 0.5 ppb level was less complete.

Various amounts of aflatoxin B_1, B_2 and M were found in the livers and kidneys as well as traces in heart, muscle and adipose tissue of pigs fed diets 2 and 3 (*Tables 4* and *5*).

Tables 6 and *7* summarize the residues detected in organs and carcasses which would pass the meat inspection.

Table 6. Residues in liver and kidney

	Diet 2		Diet 3	
	mean	max.	mean	max.
	(ppb $B_1 + B_2$)			
Liver, accepted at meat inspection	8	23	22	54
Kidney	6	20	10	53

Table 7. Residues in other tissues

Tissue	Percentage of samples containing traces*	
	Diet 2	Diet 3
Heart	5	22
Muscle	10	50
Adipose	35	50

*trace = less than 1 ppb.

Among the four control pigs previously mentioned, three contained no aflatoxin in the organs and tissues investigated. The liver of the fourth pig contained traces of M-toxin, estimated on t.l.c. plates by comparison with a qualitative standard of M-toxin. Other organs and tissues contained no detectable aflatoxin.

Results were confirmed by chemical tests, including derivative formation[6] and ultraviolet spectroscopic examination of B_1 purified by thin-layer chromatography[7]. Bioassay confirmation included tests based on *B. megaterium* inhibition[8], chicken-embryo toxicity[9] and duckling bioassay[10]. All these tests confirmed the presence of aflatoxin, especially B_1.

Bioassay including the chicken-embryo toxicity test and the duckling test, using extracts of the liver of one control pig, showed no toxicity reaction.

Thus it may be concluded that bacon pigs containing sizeable amounts of aflatoxin at slaughter are able to pass meat inspection because macroscopical alterations of organs and carcass are slight.

A similar problem has been encountered when ochratoxin-contaminated feed is used for bacon pigs[11]. This calls for careful control of animal feed, in order to avoid the use of heavily contaminated batches, because mycotoxins will be partly transmitted through slaughter animals into the human food channel.

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