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TIME-DEPENDENT DISAPPEARANCE OF OCHRATOXIN A RESIDUES IN TISSUES OF BACON PIGS

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SUMMARY

Crystalline ochratoxin A was administered to bacon pigs for one month. After termination of toxin exposure the pigs were slaughtered at different intervals and analyses for ochratoxin A residues in four tissues were conducted. Kidneys contained the highest concentrations, and fat the lowest, at each interval. Ochratoxin A disappeared from muscles and fat after 2 weeks, from liver after 3 weeks, and from the kidneys after 4 weeks. The toxin disappeared from tissues exponentially. All the pigs would have passed the meat inspection because no pathologic lesions were developed although tissues contained mycotoxin residues. The results of this study indicate that contamination of meat by ochratoxin A may be avoided by feeding pigs ochratoxin-free feed during the last 4 weeks before slaughter.

INTRODUCTION

Ochratoxin A, a dihydro-isocoumarin derivative linked through its 7-carboxy group to L-β-phenylalanine, is a nephrotoxic secondary metabolite of several fungal species included in the genera Aspergillus and Penicillium. This mycotoxin has been found as a natural contaminant of plant products, especially cereals, in many countries [1], and has been observed as a causal

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determinant of porcine nephropathy, a naturally occurring disease in pigs [2–3].

In an experimental reproduction of porcine nephropathy employing ochratoxin A-contaminated cereal feed, residues of ochratoxin A were detected in various tissues of bacon pigs, including kidney, liver, muscular tissue and fat [3]. All the carcasses of these experimental bacon pigs, and all organs except some kidneys would have passed the meat inspection, because the only lesions present were nephropathy, in a degree not demanding condemnation. Thus food of animal origin, contaminated with ochratoxin A residues, may pass meat inspection and enter the human food channel. Ochratoxin is not destroyed completely by heat treatment (autoclaving) [4]. Although toxicological evaluations of ochratoxin A in humans have not yet been undertaken, this compound can undoubtedly be categorized as undesirable in human foods. Residues of ochratoxin A in bacon pigs may therefore represent a public health problem.

One way of avoiding residues in slaughter animals would be the use of non-contaminated feed throughout the feeding period. However, this may be impossible since control measures covering all feed lots would be difficult to implement. The use of ochratoxin A-contaminated feed during the main part of the feeding period may be acceptable provided that the animal tissues are toxin-free at slaughter. This effect might be implemented by changing to controlled, non-contaminated feed during the last part of the feeding period before slaughter. The duration of this last part of the feeding period can be specified provided the disappearance rate of ochratoxin A residues is established. This study was undertaken to elucidate the disappearance rate of ochratoxin A residues after termination of exposure.

MATERIALS AND METHODS

Experimental animals

Five blocks (litters) of SPF pigs, each comprising 5 females of Danish Landrace, were purchased at about eight weeks of age. Within the blocks the 5 animals were distributed into 5 groups. During the experimental period the pigs were fed twice a day, kept in individual pens with concrete floor and straw as bedding, and maintained at a temperature of 18° C. Body weight was recorded every week. A full description of the experimental procedure has been published [5]. The composition of the feed was as follows (in percentage): barley: 73.6; soybean meal: 24.0; calcium carbonate: 0.8; dicalcium phosphate: 1.1; sodium chloride: 0.4; vitamin-trace mineral mixture: 0.1.

Ochratoxin

Autoclaved cracked wheat (40% moisture) was inoculated with *Aspergillus ochraceus* NRRL 3174 and incubated at 25° C on a rotary shaker for 10 days. The mouldy wheat (1 kg) was extracted with chloroform (1 l) and subsequently the extract was mixed with an equal volume of 0.5 M $NaHCO_3$. The aqueous bicarbonate layer was separated from the chloroform and

TABLE I

EXPOSURE LEVELS OF OCHRATOXIN A

Time period	Body weight (kg)	Exposure of ochratoxin A (μg per kg body weight)
1st week	20	43
2nd week	25	42
3rd week	30	41
4th week	35	40

acidified to pH 2.0. This solution was extracted three times with 200 ml volumes of chloroform. The chloroform solution was evaporated to 20 ml and added to a silicic acid column prepared in benzene. Initial elution was carried out with benzene followed by elution of the ochratoxin with benzene : acetic acid (85 : 15). Ochratoxin was crystallized three times from benzene and dried under vacuum. The yield was approx. 0.25 g of crude ochratoxin per kg of wheat. The crystalline preparation contained 90% ochratoxin A and 5% ochratoxin B, as determined by thin-layer chromatography.

Ochratoxin A exposure

The crystalline toxin preparation was mixed with lactose and distributed in gelatine capsules (0.5 ml). Group one, the control group, was given capsules containing only lactose. Each pig in groups 2—5 was given one capsule perorally every afternoon for one month. The amount of ochratoxin A in the capsules was graded according to the body weight, as indicated in Table I, so that the exposure corresponded to a feed level of approx. 1 ppm, an average level observed in naturally contaminated cereals associated with mycotoxic porcine nephropathy [2].

After one month the ochratoxin A exposure was terminated and pigs were fed the toxin-free diets for various intervals, as indicated in Table II. For group one this period lasted from 1 to 6 days. The feed used throughout the study was analysed regularly for ochratoxin according to Nesheim et al. [6].

TABLE II

TIME LAPSE AFTER TERMINATION OF OCHRATOXIN A EXPOSURE

Group 2	1 day
Group 3	1 week
Group 4	2 weeks
Group 5	4 weeks

Pathology

After the various intervals the pigs were anesthetized with intravenously injected Narcodorm®, exsanguinated by cutting the carotids, and necropsy was performed on all tissues. Representative sections of most organs and tissues were fixed in neutral buffered 10% formalin. Sections of kidney were processed for paraffin embedding, sectioned at 4 μm and stained with Harris hematoxylin-eosin, iron hematoxylin (F.C.C. Hansen-van Gieson), and periodic-acid-Schiff (PAS).

Residues of ochratoxin A

50-g samples of kidney, liver, muscular tissue (from the foreleg), and fat (abdominal adipose tissue) were analysed for ochratoxin according to a previously published method [3]. This method was based upon a thin-layer chromatography procedure [6], followed by ammoniation [7], before densitometric quantitation was performed. The method has a lower level of detection at 1—5 ppb. Positive findings of ochratoxin A were confirmed by derivative formation [6].

Statistical evaluation

Results are expressed as means ± S.E.M. Coefficients of the equations for disappearance were estimated by the least-square technique, and regression analysis was carried out [8].

RESULTS

Pathology

At necropsy no significant lesions were observed. One pig from group 1 showed catarrhal bronchopneumonia in both intermediate lobes, and a pig from group 2 demonstrated a chronic peritonitis of the spiral colon.

Microscopical examination of tissues showed no significant lesions. In the kidneys the glomeruli appeared normal in all 5 groups, and no significant differences were noted in the height of the brush border of the proximal tubules. The basement membranes appeared equally normal. The remaining parts of the nephrons, the vascular system, and the interstitial tissue appeared morphologically normal.

In a number of pigs derived from all 5 groups leucocytic foci were found located interstitially in the cortex and the medulla.

Residues of ochratoxin

No ochratoxin was found in any tissue of the control pigs (group 1). The highest concentrations of ochratoxin A were observed in group 2, as expected (Table III). Toxin distribution in tissues (of group 2) is shown in Fig. 1; increasing concentrations were observed from fat, muscle, liver, to kidney. The same distribution pattern was found in the other groups (3 to 5). Ochratoxin A disappeared from tissues exponentially. The exponential equations for disappearance in all 4 tissues are listed in Table IV. The half-life of residues, cal-

TABLE III

OCHRATOXIN A RESIDUES IN TISSUES AT VARIOUS INTERVALS AFTER TERMINATION OF EXPOSURE

Tissue	Group	Time after termination of exposure (days)	Means ± S.E.M. of ochratoxin A (μg/kg)
Kidney			
	2	1	25.70 ± 3.22
	3	8	7.66 ± 0.71
	4	15	3.02 ± 0.49
	5	29	0.54 ± 0.35 [a]
Liver			
	2	1	17.80 ± 3.17
	3	8	5.14 ± 0.22
	4	15	0.58 ± 0.58 [a]
	5	29	ND
Muscles			
	2	1	11.54 ± 1.92
	3	8	2.22 ± 0.10
	4	15	ND
	5	29	ND
Fat			
	2	1	5.95 ± 2.54
	3	8	2.54 ± 0.93 [a]
	4	15	ND
	5	29	ND

ND, No detectable amounts.
[a] Calculations based on data, which included one or more ND, treated as zero.

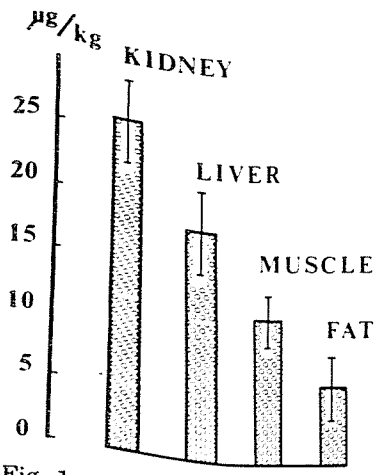

Fig. 1. Distribution of ochratoxin A residues in tissues one day after termination of toxin exposure. Each bar represents the mean ± S.E.M. of 5 pigs (Group 2).

TABLE IV

EQUATIONS FOR RESIDUE DISAPPEARANCE

Kidney	$C = 28.22 \exp(-0.1522\ t)$
Liver	$C = 19.49 \exp(-0.1598\ t)$
Muscular tissue	$C = 12.94 \exp(-0.2096\ t)$
Fat	$C = 4.62 \exp(-0.0565\ t)$

C, concentration of ochratoxin A (μg/kg).
t, time (days) after termination of ochratoxin A exposure.

TABLE V

RESIDUAL LIFE (RL_{50}) CALCULATED FROM THE EXPONENTIAL EQUATIONS

RL_{50} for fat was impossible to calculate because ochratoxin A was indetectable at the last 2 test periods

Tissue	RL_{50} (days)
Kidney	4.5
Liver	4.3
Muscles	3.3

TABLE VI

LINEAR REGRESSION OF OCHRATOXIN A IN VARIOUS TISSUES ON KIDNEY CONTENT

Linear regression equation: $y = a + b \cdot x$; x, kidney concentration of ochratoxin A (μg/kg).
r, correlation coefficient.

Tissue	Equation	Standard error of regression coefficient	Standard of "y" for $x = 0$	r
Liver	$y = -0.650 + 0.706 \cdot x$	0.706 ± 0.060	0.650 ± 1.535	0.937
Muscular	$y = -0.603 + 0.438 \cdot x$	0.438 ± 0.052	0.603 ± 1.324	0.888
Adipose	$y = 0.775 + 0.309 \cdot x$	0.309 ± 0.047	0.775 ± 1.215	0.739

culated from the exponential equations, are shown in Table V, indicating RL_{50} values in the range of 3—5 days.

Ochratoxin A concentrations were below detection level in fat and muscular tissue after 2 weeks, and after 4 weeks only the kidneys contained detectable concentrations (in 2 out of 5 animals). Ochratoxin B, and ochratoxin α, a metabolic product of ochratoxin A, were not detected in any tissue.

Correlation was observed between ochratotoxin A residues in three tissues (liver, muscle, fat) on kidney concentrations (Table VI).

DISCUSSION

The pigs in this study were slaughtered at a bodyweight of approx. 40 kg, compared to the normal body weight of bacon pigs at slaughter, 90 kg. It is assumed, however, that the use of younger test animals does not influence the results significantly.

The magnitude and distribution pattern of residues one day after termination of toxin exposure (group 2) is similar to previous observations encountered in an experimental study [3] employing continued exposure throughout the feeding period.

The disappearance rates show that a period of 4 weeks on an ochratoxin-free feed before slaughter will essentially eliminate ochratoxin A residues from bacon pigs. All the pigs would have passed meat inspection because no pathological lesions were developed during 1 month exposure to diets containing 1 mg per kg feed. This is in accordance with the results of an earlier study [3], in which animals exposed to 1 ppm even for 2 months did not develop nephropathy. It should be stressed, however, that residues detected in this study are unmetabolized ochratoxin A as identified on TLC plates. In a study in rats [9] employing [^{14}C]ochratoxin A, labeled in the phenylalanine moiety it was observed that only 30 to 40% of the total radioactivity in kidney and liver of 24-h samples was ochratoxin A. The authors concluded that ochratoxin A must convert to some as yet unknown compounds in rats.

The kidneys contained, in all groups, the highest residue concentration. Kidneys may therefore be proposed as an indicator tissue for use in meat inspection. The equations in Table VI allow the estimation of ochratoxin A residues in the remaining part of the slaughter animal, so that the final judgement by the meat inspection can be arrived at when only the kidney value is monitored.

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