Vet.Med.J.,Giza. Vol.51, No.2. (2003) :223-234.

MONITORING OF AFLATOXINS RESIDUES IN EDIBLE BY-PRODUCTS OF CATTLE

E.S.E. SHABANA, EMAN M.S. ZAKI and ZEINAB M. NIAZI
Animal Health Research Institute. Dokki- Giza

Received: 31.12. 2002.
Accepted:9. 3. 2003.

SUMMARY

A total of 80 random samples of cattle edible by-products represented by 20 each of tongue, lung, liver and small intestine half of them were fresh and the other half were cooked, were collected from different butcher shops, vendors and shops of ready to eat edible animal by- products (Masmat) in Giza governorate. The samples were analyzed for presence of aflatoxins residues. The obtained results indicated that 4(40%); 2(20%); 3 (30%) and 4(40%) of fresh samples and 5(50%); 3(30%); 1 (10%) and 2(20%) of cooked samples of tongue, lung, liver, and small intestine respectively, were contaminated with aflatoxins. The highest mean value was estimated in cooked lung samples (aflatoxin B_1 6.240± 5.194 ppb), while the lowest mean value was determined in fresh tongue (aflatoxin B_1 1.573±0.635 ppb).

INTRODUCTION

Edible by- products are defined as animal meat products, other than skeletal muscle that are eaten, such as tongue, lung, liver and small intestine, and are considered highly nutritive and attractive food article. They are consumed in Egypt on a large scale after being employed in a large number of dishes and cooking by different methods.

Mycotoxins are toxic products secreted by microscopic fungi. They can contaminate products of vegetal origin consumed by humans and cattle, therefore secondary by humans. The most important mycotoxins are aflatoxins which are produced by *Aspergillus flavus* and *Asp. parasiticus* growing on food (e.g. nuts, cereals, oily seeds and beans) under favorable temperature and humidity conditions, before or during harvest or improper storage (Ioannou-Kakouri et al., 1999 and Pitt, 2000).

Aflatoxins (B_1, B_2, G_1 and G_2) possess acute and chronic toxicity at low concentration and are potent mutagenic, carcinogenic, or teratogenic to a wide range of organisms. Also aflatoxins (M_1

and M_2) possess the same toxicity as the aflatoxins from which they were derived (AFs B_1 and B_2). Moreover, the International Agency for Research on Cancer classified aflatoxin B_1 as a probable human carcinogen (Dichter, 1984; Garner, 1984; IARC, 1987; Newberne, 1987 and Park, 1995).

Mycotoxins are heat stable and are not destroyed by heating to ordinary cooking temperature of food, so, in order to achieve destruction of aflatoxins, temperature in excess of 300°C are required (Bullerman, 1987).

Several reviews have summarized aflatoxin B_1 and M_1 concentrations detected in edible meat tissues, milk and eggs. In general, a major portion of the ingested aflatoxin is excreted in animal wastes within 24 hr., and small quantities are retained in tissues, therefore quantitation of the parent toxins and potentially toxic metabolites are important in the evaluation of the food safety (Rodricks and Stoloff, 1977; Edds, 1979; Stoloff, 1979; Gregory and Manley, 1982).

Thus, the present study was designed for monitoring aflatoxins residues in edible by-products of cattle.

MATERIAL AND METHODS

Eighty random samples of cattle edible by-products represented by twenty each of tongue, lung, liver and small intestine, half of these samples were fresh while the other half were cooked, were collected from different butcher shops, vendors and shops of ready to eat edible animal by- products (Masmat) in Giza governorate. Each sample was collected individually in clean and dry polyethylene bags and transfered in ice box to the laboratory and kept frozen until analysed for the presence of aflatoxins (AFs) residues.

Analysis of aflatoxins:

Aflatoxins were analysed by a thin layer chromatography (TLC) method according to the technique recommended by AOAC (2000) as following:-

Preparation of sample extract and clean up: 100g of the finely homogenized samples were throughly mixed with 10 ml citric acid solution (20%) in 500 ml Erlenmeyer flask. After 5 min stiring again and mixed with 20 g diatomaceous earth. The mixture was then extracted with 200 ml CH_2Cl_2 on a wrist - action shaker for 30 min. The mixture was filtered through fast flow paper into 300 ml Erlenmeyer flask containing 10g Na_2SO_4 (AR grade - anhydrous), then the flask was swirled gently for 2 min and filtered the content through medium flow paper into a 250 ml measuring cylinder and the volume was recoreded. The filtrate was evaporated in 500 ml round-bottom flask to near dryness. Slowly, the slurry (50 ml CH_2Cl_2+ 2 g Silica gel G(Merck) activated by drying 1h at 105°C) was transferred to a glass

olumn and 2 g $Na_2 SO_4$ was added to give a 2 m upper layer. The concentrated filterate was re-dissolved in 25 ml CH_2Cl_2 and allowed to percolated through the column, then washed successively with 25 ml toluene - Ch_3COOH (9+1), 25 ml hexane and 25 ml hexane -ether- CH_3CN (6+3+1) and discard washes. AFs were eluted with 40 ml CH_2Cl_2- acetone (4+1) and evaporated to near dryness on steam bath, then transferred to a small vial with a good stopper and evaporated to dryness under reduced pressure at 40 - 50°C and save for TLC.

TLC plates: Standard 20 cm x 20cm glass plates coated with a layer (0.5 mm) of silica gel (Merck, allowed to dry in air, then activated by heating 2h at 80°C in a hot oven and removed immediately to a desiccotor to cool, were employed in these study.

Aflatoxin reference standard: Pure standard mixtures of AFs giving concentration at final dilution equal to 0.5µg AFs B_1, G_1, and M_1/ml and 0.1 µg for AFs B_2, G_2 and M_2/ml $CHCl_3$ (Sigma Chemical Co.; Supleco Inc.) were employed in these examined.

Devlopment of the chromatogram: Each residue from tested samples was dissolved in 100 µl $CHCl_3$ and 20 µl was spotted on TLC paltes (Silica gel G); 10 µl AFs standards were also spotted on the TLC plates using micropipettes, at 2 cm from the bottom of a TLC plate. Then the TLC paltes were palced in developing tank and allowed to run for 16 cm from the baseline in a solvent of $CHCl_3$- acetone (9+1). Dried paltes were examined under UV light (366 nm). AFs concentrations were determined by comparing the R_f and intensity of fluorescence of the AFs standards with unknown samples. Positive samples were further confirmed by reaction with trifluoroacitic acid - hexane spray (1 + 4) in accordance with conditions approved by the AOAC (2000).

Calculation: The concentration of AF was calculated from the equation.

$$\mu g AF/ Kg = (S_x Y_x V) / (X_x w)$$

where: S=µlAF reference standard spot equal to unknown; Y= concentration of reference standard (µg/ml); V=µl of final dilution of sample extract; X=µl sample extract spotted giving fluorsecent intensity equal to S; W= (100 g x filtrate volume)/ 200.

RESULTS AND DISCUSSION

Edible tissue by its very nature is free of toxic substance. It can however, contain unwanted foreign substance as a consequence of human activities (Wild, 1997). AFs are considered to be one of the most dangerous toxins naturally contaminating food and feed stuffs. The possible presence of its residues in edible tissues after ingestion of contaminated feed by farm animals is well documented (Shotwell et al., 1980).

The data presented in table (1) revealed that the percentage of positive samples of cattle edible by-prodcuts harbouring AFs residues were 40%, 20%, 30% and 40% for fresh samples, while were 50%, 30%, 10% and 20% for cooked samples of tongue, lung, liver and small intestine, respectively.

The obtained results for the tongue, lung and small intestine samples were supported by the results obtained by Richard et al. (1983), who pointed out that AFs B_1 and M_1 could be detected in the lung and other edible tissue Samples of Holstein- Friesian steers which were fed on a diet naturally contaminated with AFs B_1 and B_2. Moreover, Stubblefield et al. (1983) determined AFs B_1 and M_1 residues in tongue, lung, liver, small intestine and other tissue samples of Holstein cows which had been fed a diet contaminated with 0.35 mg AF B_1/ kg of body weight for 3 consecutive days.

The foregoing data of liver samples were in agreement with the previous results of Sayed et al. (2000). Comparatively lower percentage were recorded by Dhavan and Choudary (1995) & Hammad (1995), while higher percentage were recorded by El-gazzar (1997) and Shabana (1999). On the other hand, Zaki et al. (1993) failed to detect any level of AFs from liver, kidney and muscles of the native breed bulls.

The recognition of AFs residues in the cooked samples could be attributed to the fact that AFs are heat stable at the range of temperature usually employed in the cooking and /or processing of foods (Aibara, 1978; Furtado et al., 1981; El-Shawaf, 1990; Shabana, 1999 and El-Zeini, 2001).

Table (1): Percentage of aflatoxins residues in individual cattle edible by-product samples

Examined samples	No.of examined samples	No. of samples containing aflatoxin residues			
		Fresh		Cooked	
		No.	%	No.	%
Tongue	10	4	40	5	50
Lung	10	2	20	3	30
Liver	10	3	30	1	10
Small intestine	10	4	40	2	20

Table (2): Distribution of aflatoxins residues in individual cattle edible by - products sampes.

No. of smaples	Fresh				Cooked			
	Tongue	Lung	Liver	Small intestine	Tongue	Lung	Liver	Small intestine
1	B_1	-	-	B_2	G_1 G_2	B_1	-	B1
2	-	B_1 B_2	-	M1	-	B_1	-	-
3	B_1 B_2	-	-	-	-	-	-	-
4	-	-	B_1 B_2 G_1	-	-	-	-	-
5	-	-	-	B_1	M_1 M_2	-	-	-
6	-	-	B_1 B_2	-	M_1 M_2	-	-	-
7	B_1 B_2	-	-	-	B_1 B_2	B_1 B_2 G_1 G_2	-	-
8	-	-	-	B_1 B_2	-	-	B_1 B_2 M_1	-
9	-	B_1	-	-	-	-	-	-
10	M_1 M_2	-	M_1 M_2	-	M_1	-	-	B_1 B_2 G_1 G_2

The results achieved in table (2) illustrated the distribution of AF residues in individual fresh and cooked cattle edible by product samples, which were nearly similar to the results recorded by Richard et al. (1983); Stubblefield et al. (1983); Hammad (1995); Shabana (1999) and Sayed et al. (2000).

The summarized data in table (3) showed the incidence of AF residues in individual fresh and cooked edible cattle by- product samples. Similar findings were reported by Dhavan and Choudary (1995) and El- Shewy et al. (1997). Where as by El-gazzar (1997) and Sayed et al. (2000), recorded higher figures.

In this regard, Stubblefield et al., (1983) and Trucksess et al., (1983) stated that there is an evidence that the toxin could enter the blood directly through the rumen epithlium and were then available for distribution to the other tissues. Another contributory factor could be the reabsorption of the toxins from the bile into the small intestine, and then back to the blood.

In case of fresh samples as indicated in table (4), the total AFs in tongue samples varied from 0.625 to 5.556 ppb the highest amount of individual AFs was 2.778 ppb for AFs B_1 and B_2 and 2.273 ppb for AF M_1 but 1.515 ppb for AF M_2. While the total AFs in lung samples ranged from. 2.282 to 7.000 ppb, the highest level of individual AFs was 7.000 ppb for AF B_1 and 1.191 ppb for AF B_2. On the other hand, the total AFs in liver samples varid from 13.720 to 16.660 ppb, the highest amount of individual AFs was 6.860 ppb

Table (3): Incidence of aflatoxins residues in individual cattle edible by products samples.

samples	Fresh												Cooked											
	No. of samples	+ve samples	B_1		B_2		G_1		G_2		M_1		M_2		No. of samples	+ve samples	B_1		B_2		G_1		G_2		M_1		M_2	
			+ve	%	+ve	%	+ve	%	+ve	%	+ve	%	+ve	%			+ve	%	+ve	%	+ve	%	+ve	%	+ve	%	+ve	%
Tongue	10	4	3	30	2	20	-	-		-	1	10	-	-	10	5	1	10	1	10	1	10	1	10	3	30	2	20
Lung	10	2	2	20	1	10	-	-		-	-	-	-	-	10	3	3	30	1	10	-	-	1	10	-	-	-	-
Liver	10	3	2	20	2	20	-	-		-	1	10	1	10	10	1	1	10	1	10	-	-	-	-	1	10	-	-
s.intestine	10	4	2	20	2	20	-	-		-	1	10	-	-	10	2	2	20	1	10	1	10	1	10	-	-	-	-

Table (4): Levels of aflatoxins residues (µg/kg) in individual cattle edible by-products samples.

examined samples	Fresh								Fresh							
	samples No.	B_1	B_2	G_1	G_2	M_1	M_2	Total	samples No.	B_1	B_2	G_1	G_2	M_1	M_2	Total
Tongue	1	0.625	-	-	-	-	-	0.625	1	-	-	2.583	2.583	-	-	5.166
	3	1.316	1.316	-	-	-	-	2.632	5	-	-	-	-	2.273	2.273	4.546
	7	2.778	2.778	-	-	-	-	5.556	6	-	-	-	-	1.515	1.515	3.130
	10	-	-	-	-	2.273	1.515	3.788	7	2.778	2.778	-	-	-	-	5.550
	-	-	-	-	-	-	-		10	-	-	-	-	1.515	-	1.515
Lung	2	1.191	1.191	-	-	-	-	2.282	1	0.844	-	-	-	-	-	0.844
	9	7.000	-	-	-	-	-	7.000	2	1.250	-	-	-	-	-	1.250
	-	-	-	-		-	-		7	16.625	16.625	16.625	16.625	-	-	66.500
Liver	4	4.762	4.762	4.762	-	-	-	14.286	8	1.250	1.195	-	-	0.844	-	3.289
	6	6.680	6.860	-	-	-	-	13.720		-	-	-	-	-	-	
	10	-	-	-	-	8.330	8.330	16.660		-	-	-	-	-	-	
Small intestine	1	0.444	-	-	-	-	-	0.444	1	1.195	-	-	-	-	-	1.195
	2	-	-	-	-	2.000	-	2.000	10	6.860	6.860	0.444	0.444	-	-	14.608
	5	7.000	-	-	-	-	-	7.000		-	-	-	-	-	-	-
	8	7.000	2.000	-	-	-	-	9.000		-	-	-	-	-	-	-

Table (5): Analysis of the level of aflatoxins residues (µg/ kg)* in the examined cattle edible by- products samples

Samples		Aflatoxin contamination levels																					
		Fresh								Cooked													
		B1		B2		G1		G2		M1		M2		B1		B2		G1		G2		M1		M2	
		min	Max	min	Max	min	Max	min	Max	min	Max	min	Max	Min	Max	min	Max	min	Max	min	Max	min	Max	min	Max
Small intestine		0.444	7.000	2.000						2.000				1.195	6.860	6.860		0.444		0.444					
Liver		4.762	6.860	4.762	6.860	4.762				8.330		8.330		1.250		1.195						0.844			
Lung		1.191	7.000	1.191										0.844	16.625	16.635		16.625		16.625					
Tongue		0.625	2.778							2.273		1.515		2.778		2.778		2.583		2.583		1.515	2.273	1.894	0.379
Samples	B1 S.E.**	2.185	4.815	2.000																					
	B1 mean	1.049	5.811	5.811						8.330		8.330		1.250		1.195						0.844			
	B2 S.E.	0.635	1.573	1.049																					
	B2 mean	2.905	4.096	1.191										0.844	16.625	16.635		16.625		16.625					
	B2 Max	1.191	7.000							2.273		1.515		2.778		2.778		2.583		2.583		1.515	2.273	1.894	0.379

*µg/Kg= ppb

** SE= Standard Error

for AFs B_1 and B_2 but 4.762 ppb for AF G_1 and 8.330 ppb for AFs M_1 and M_2. In addition to, the total AFs in small intestine samples ranged from 0.444 to 9.000 ppb the highest level of individual AFs was 7.000 ppb for AF B_1, but 2.000 ppb for AFs B_2 and M_1. In case of cooked samples, the total AFs in tongue samples varied from 1.515 to 5.556 ppb in case of AFs B_1 and B_2 the highest amount estimated was 2.778 ppb and 2.583 ppb for AFs G_1 and G_2 also 2.273 ppb for AFs M_1 and M_2. On the other hand, the total AFs in lung samples ranged from 0.844 to 66.500 ppb and the highest level of individual AFs B_1, B_2 G_1 and G_2 was 16.625 ppb. Only one liver sample had a total AFs of 3.289 ppb. Moreover the total AFs in small intestine samples varied from 1.195 to 14.608 ppb. The highest level of individual AFs was 6.860 for B_1 and B_2 but 0.444 for AFs G_1 and G_2.

The above obtained results were lower than those reported by El-Shewy et al. (1997); Shabana (1999) and Sayed et al. (2000), while higher than that recorded by Helferich et al. (1982); Richared et al. (1983); Stublefield et al. (1983); Hammad (1995) and El-gazzar (1997). This variation in results may be due to the fact that the level of AFs contamination in feeds stuffs is important factor influencing the AF residues in various tissue. However, Trucksess et al. (1983) suggested that variation in toxin concentration may be related to rumen motility which could affect the rate of toxin absorption to the blood.

It is of important to emphasise that only one sample of each of fresh liver and cooked lung were contaminated with more than the maximum tolerated levels of total AFs 15 ppb reported by WHO (Jelinek et al. 1989). If we consider AF B_1 only, which is the most carcinogenic one, it was found that only one sample of each of fresh lung and liver, and 2 samples of fresh small intestine, also only one each of cooked lung and small intestine samples exceed permissible limit of AF B_1 5 ppb according to FAO (1997).

Mountney and Gould (1988) stated that the consumption of low levels of mycotoxins over extended periods constitute a public health hazards as many mycotoxins have been shown to be carcinogenic. Where as FDA (1977) stipulated that the daily intake of AF by a person weighing 50 kg should be kept below 0.5 ng.

As demonstrated in table (5) the highest mean value of AFs B_1 and B_2 residues were estimated in fresh liver samples 5.811±1.049 for each, while AFs G_1, M_1 and M_2 were detected in one sample thus they were not included in the analysis, followed by 4.815± 2.185 for AF B_1 in small intestine then 4.096± 2.905 for AF B_1 in lung samples finally 1.573± 0.635 for AF B_1 in tongue.

In case of cooked samples, the highest mean value of AF residues, AF B_1 was determined in lung samples 6.240± 5.192 followed by 4.028± 2.833

in small intestine samples then 1.894± 0.379 for each of AF M_1 and M_2 for tongue samples.

From above results, it is clear that the quantitated AF residues in fresh edible by- products samples were greater than those obtained by Richard et al. (1983); Stubblefield et al. (1983); Dhavan and Choudary (1995); Hammad (1995) and El-gazzar (1997), while lower than those reported by El-Shewy et al. (1997); Shabana (1999) and Sayed (2000).

Conclusion & Recommendations

Many pubic health hazard may be developed as a result of AF residues. From all the above results which showed the occurrence of AF residues with relatively high percentages and levels in different fresh and cooked edible tissue of apparently sound may be among the factors responsible for the outbreak of cancer in Egypt. Furthermore, AFs damage the liver, kidney and thymus producing a variety of effect including disruption of carbohydrate, protein and lipid metabolism, immuno-suppression, decreased growth rate and lowered productivity, which become apparent after the accumulation of lesions due to chronic ingestion of very low levels of toxin over long period (Cheeke and Shull 1985).

Thus, to provide the consumers with safe edible tissues "free or nearly free from AF residues". The following suggestive measures should be fulfilled:-

1- **In animal feed factories:** strict control measures should be applied to assure that it marketed feeds and its related feed stuffs which intended for feeding of dairy and fattening animal, are free or nearly free from AF residues.

2- **Before mass slaughtering of any animal species,** meat and any edible tissues from a few representative number of apparently healthy animal in question must be analysed for occurrence of AF residues. Where any level of contamination be detedcted, the slaughter should be delayed. Richard et al. (1983) stated that no detectable residues in the meat and tissues examined in the fattening steers (fed corn free of AF) 2.5 weeks after 15 weeks feeding of contaminated feed of approximately 455 ng of AFB1/g. Thus discontinuance of dietary AF in fattening animals for a period before slaughter appears to bean effective means of attaining meat free of AF residues.

REFERENCES

Aibara, K. (1978): "Aflatoxin: investigation on traditional foods and imported food stuffs in Japan". In: Toxicology, Biochemistry and Pathology of Mycotoxins. (Urgnchi, K. and Yamazki, M. Ed). Kodanska Ltd., Japan.

Association of Official Analytical Chemistis "AOAC" (2000): "Official methods of analysis of AOAC International, Natural toxins" ed. W. Horwitz, 17th Ed. Chapter 49,p.1-64 Gaithersburg, Maryland 20877-2417 USA.

Bullerman, L. B.(1987): Methods for detecting mycotoxins in food and beverages in food and beverage mycology 2nd Ed. By Beauchate, L.R. Van Nostrand Reinhold. New York.

Cheeke, P.R. and Shull, L.R. (1985): "Natural toxicants in feeds and poisonous plants". Avi publishing Company, Westport, CT. 393-476.

Dhavan, A.S. and Choudary, M. R. (1995): "Incidence of aflatoxins in animal feed stuffs: A Decade's Scenario in India", J. AOAC International 78(3): 693-698.

Dichter, C. R. (1994): "Risk estimates of liver cancer due to aflatoxin exposure from peanuts and peanut products". Food and Chemical Toxicology 22: 431-437.

Edds, G.T. (1979): "Aflatoxins produced by *Aspergillus flavus* and *Aspergillus parasiticus* in feed, on food, causing toxicity in livestock and poultry", p. 80-164. In W. Shimoda (ed.) Conference on mycotoxins in animal feed grains related to animal health. U.S Department of commerce, National Technical Information Service, Spring field, VA.

El-Gazzar, M.M.M. (1997): "Aflatoxin residues in meat and edible offals". J. Egypt Vet. Med. Ass. 57(1): 203-214.

El- Shawaf, A. M. (1990): "Microbial studies on aflatoxins in meat products". Ph. D.Thesis Fac. Agric. Mansoura Univ. Egypt.

El- Shewy, E. A.; Ashoub, M. A. and El-Hoshy, S.M. (1997): "Toxicity of aflatoxin, ochratoxin and their residues in some animal products". Alex. J. Vet. Sci. 13 (3): 219- 231.

El-Zeini, S. (2001): "Mycotoxins residues in some Egyptian poultry meat products". Vet. Med. J. Giza 49(4): 591-598.

Food and Agriculture Organization of the United Nations "FAO" (1997): "World wide regulations for mycotoxins 1995". A Compendium 64: 7-28.

Food and Durg Administration "FDA" (1977): Fed Regist 42 (35): 1042. Cited after Hsieh, D.P.H. (1983): Metabolism and transmission of mycotoxins. Proc. Int. Symp Mycotoxins P. 151-165.

Furtado, R.M.; Pearson, A.M.; Gray, J.I.; Hogberg, M.G. and Miller, E. R. (1981): "Effects of cooking and/ or processing upon levels of aflatoxins in meat from pigs fed a contaminated diet". J. Food Science 46: 1306-1308.

Garner, R.C. (1984): "Liver cancer", New scientist. 102. (1410): 52 (Cited from Review of Medical and Vet. Mycology. 1984, 19, 170, Abst. No. 1316).

Gregory, J. F. and Manley, D. B. (1982): "High performance liquid chromatographic quantitation of aflatoxin metabolites in animal tissues" J. AOAC 65(4): 869-875.

Hammad, A. E. M. (1995): "Characterization of the fungi and aflatoxins isolated from frozen meat". Ph. D. thesis Fac. Vet. Med. Alexandria Univ. Egypt.

Helferich, W.C.; Garrett, W.N.; Hsieh, D. P.H; and Baldwin, R.L. (1982): "Feedlot performance and tissue residues of cattle consuming rations containing aflatoxins" J. Animal Science 55 (supple 1): 329-330.

International Agency for Research on Cancer "IARC" (1987): "Monograph on the evaluation of carcinogenic risk to humans". Suppl. 1, IARC, Lyon, France. 82- 87.

Ioannou- Kakouri, E.; Aletrari, M.; Christeu, E.; Hadjioannou-Rath, A.; Koliou, A. and Akkelidou, D.(1999) "Surveillance and control of aflatoxins B_1, B_2, G_1, G_2 and M1 in food stuffs in the republic of Cyprus 1992-1996." J. AOAC International 82(4): 883-892.

Jelinek, C.F.; Pohland, A.E. and Wood, G. E. (1989): "Worldwide occurrence of mycotoxin in foods and feeds an update". J. AOAC 72: 223-230.

Mountney, G., and Gould, W. (1988): "Practical food microbiology and technology". 3rd Ed. An Avi Book Pub., New York.

Newberne, P.M.(1987): "Interaction of nutrients and other factors with mycotoxins". In: Mycotoxins in food. Pp. 177-216 (Krogh, P. ed) Academic press.

Park, D.L. (1995): Food Addit. Contam. 12, 361-371

Cited after Ioannou-Kakouri et al. (1999)

Pitt, J. I. (2000): "Toxigenic fungi and mycotoxins". Cited from Br. Med. Bull. 56 (1): 184-192.

Richard, J. L.; Pier, A.C.; Stubblefield, R.D.; Shotwell, O.L.; Lyon, R. L. and Cutlip, R.C. (1983): "Effect of feeding corn naturally contaminated with aflatoxin on feed efficiency, on physiologic, immunologic and pathologic changes and on tissue residues in steers". Am. J. Vet. Res. 44 (7): 1294- 1299.

Rodricks, J. V. and Stoloff, L. (1977): "Aflatoxin residues from contaminated feed in edible tissues of food - producing animal". In meat, milk and egg). In Mycotoxins in human and animal health. Pp. 67-79 Rodricks, J. V.; Hesseltine, C.W. and Mchllman, M.A. ed. Pathotx publishers, Inc. Park forest South. IL.

Sayed, A. M.; Mahmoud, A. - L.E. and Abou El-Alla, A.A. (2000): "Mycoflora and natural occurrence of mycotoxins in livers of imported bulls and poultry and some meat products". Assiut Vet. Med. J. 43 (86): 188-199.

Shabana, E.S.E. (1999): "Studies on mycotoxins residues in some edible animal by-products". Ph.D Thesis Fac. Vet. Med. Suez Canal Univ. Egypt.

Shotwell, O.L.; Goulden, N. L.; Hesseltine, C.W.; Dickens, J.W. and Kwolc,k, W. F. (1980): "Aflatoxins distribution in contaminated corn platnts" J. cereal Chemistry. 56: 206-208.

Stoloff, L. (1979): "Mycotoxin residues in edible animal tissues". p. 157-166. In Interactions of mycotxins in animal production. National Academy of Sciences, Washington, D.C.

Stubblefield, R. D.; Pier, A. C.; Richard, J.L. and Shotwell, O.L. (1983): "Fate of aflatoxins in tissues, fluids and excrements from cows dosed orally with aflatoxin B_1" Am. J. Vet. Res. 44 (9): 1750- 1752.

Trucksess, M. W.; Richard, J. L.; Stoloff, L.; McDonald, J. S. and Brumley, W.C. (1983): "Absorption and distribution patterns of aflatoxicol and aflatoxins B_1 and M_1 in blood and milk of cows given aflatoxin B_1". Am. J. Vet. Res. 44 (9): 1753-1756.

Wild, D. (1997): "Toxicological aspects of meat and meat products". Fleischwirtschaft 77 (9): 812-815.

Zaki, Z. M.; Shaaban, A.A.; Shehata, A. A. and Saad, M. M. (1983): "Aflatoxin residues in secretions, excretions and edible tissues of dairy and meat producing cattle". 2nd Sci., Cong. Egyptian Society for cattle diseases, 5-7 Dec., Assiut Egypt Pp. 24-33.