

MONITORING OF AFLATOXINS RESIDUES IN EDIBLE BY-PRODUCTS OF CATTLE

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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 B₁ 6.240± 5.194 ppb), while the lowest mean value was determined in fresh tongue (aflatoxin B₁ 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 (B₁, B₂, G₁ and G₂) possess acute and chronic toxicity at low concentration and are potent mutagenic, carcinogenic, or teratogenic to a wide range of organisms. Also aflatoxins (M₁

and M₂) possess the same toxicity as the aflatoxins from which they were derived (AFs B₁ and B₂). Moreover, the International Agency for Research on Cancer classified aflatoxin B₁ 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 B₁ and M₁ 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 transferred 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 thoroughly mixed with 10 ml citric acid solution (20%) in 500 ml Erlenmeyer flask. After 5 min stirring again and mixed with 20 g diatomaceous earth. The mixture was then extracted with 200 ml CH₂Cl₂ on a wrist - action shaker for 30 min. The mixture was filtered through fast flow paper into 300 ml Erlenmeyer flask containing 10g Na₂SO₄ (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 recorded. The filtrate was evaporated in 500 ml round-bottom flask to near dryness. Slowly, the slurry (50 ml CH₂Cl₂+ 2 g Silica gel G(Merck) activated by drying 1h at 105°C) was transferred to a glass

column and 2 g Na₂ SO₄ was added to give a 2 cm upper layer. The concentrated filtrate was redissolved in 25 ml CH₂Cl₂ and allowed to percolated through the column, then washed successively with 25 ml toluene - CH₃COOH (9+1), 25 ml hexane and 25 ml hexane -ether- CH₃CN (6+3+1) and discard washes. AFs were eluted with 40 ml CH₂Cl₂- 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 desiccator 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 B₁, G₁, and M₁/ml and 0.1 µg for AFs B₂, G₂ and M₂/ml CHCl₃ (Sigma Chemical Co.; Supleco Inc.) were employed in these examined.

Development of the chromatogram: Each residue from tested samples was dissolved in 100 µl CHCl₃ 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 CHCl₃- acetone (9+1). Dried paltes were examined under UV light (366 nm). AFs concentrations were determined by comparing the R_f and intensity of fluorecence of the AFs standards with unknown samples. Positive samples were further confirmed by reaction with trifluoroacetic acid - hexane spray (1 + 4) in accordance with conditions approved by the AOAC (2000).

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

$$\mu\text{gAF/ Kg} = (S_x Y_x V) / (X_x w)$$

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 fluoresecent 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-products 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 B₁ and M₁ 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 B₁ and B₂. Moreover, Stubblefield et al. (1983) determined AFs B₁ and M₁ 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 B₁/ 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 | - | B ₁ |
| 2 | - | B ₁ B ₂ | - | M ₁ | - | 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 evi-

dence 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 B₁ and B₂ and 2.273 ppb for AF M₁ but 1.515 ppb for AF M₂. 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 B₁ and 1.191 ppb for AF B₂. 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 ($\mu\text{g}/\text{kg}$) 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 ($\mu\text{g}/\text{kg}$)* 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 | mean | S.E.** | min | Max | mean | S.E. | min | Max | min | Max | min | Max | 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 | 4.815 | 2.185 | 2.000 | 4.762 | 6.860 | 5.811 | 1.049 | 4.762 | 6.860 | 5.811 | 1.049 | 4.762 | 6.860 | 5.811 | 1.049 | 4.762 | 6.860 | 5.811 | 1.049 | 4.762 | 6.860 | 5.811 | 1.049 | 4.762 | 6.860 | 5.811 | 1.049 | 4.762 | 6.860 | 5.811 | 1.049 | 4.762 | 6.860 | 5.811 | 1.049 | 4.762 | 6.860 | 5.811 | 1.049 | | | |
| Liver | 4.762 | 6.860 | 5.811 | 1.049 | 4.762 | 6.860 | 5.811 | 1.049 | 4.762 | 6.860 | 5.811 | 1.049 | 4.762 | 6.860 | 5.811 | 1.049 | 4.762 | 6.860 | 5.811 | 1.049 | 4.762 | 6.860 | 5.811 | 1.049 | 4.762 | 6.860 | 5.811 | 1.049 | 4.762 | 6.860 | 5.811 | 1.049 | 4.762 | 6.860 | 5.811 | 1.049 | 4.762 | 6.860 | 5.811 | 1.049 | 4.762 | 6.860 | 5.811 | 1.049 |
| Lung | 1.191 | 7.000 | 4.096 | 2.905 | 1.191 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | | | | |
| Tongue | 0.625 | 2.778 | 1.573 | 0.635 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | | | | |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

* $\mu\text{g}/\text{Kg} = \text{ppb}$

** SE= Standard Error

for AFs B₁ and B₂ but 4.762 ppb for AF G₁ and 8.330 ppb for AFs M₁ and M₂. 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 B₁, but 2.000 ppb for AFs B₂ and M₁. In case of cooked samples, the total AFs in tongue samples varied from 1.515 to 5.556 ppb in case of AFs B₁ and B₂ the highest amount estimated was 2.778 ppb and 2.583 ppb for AFs G₁ and G₂ also 2.273 ppb for AFs M₁ and M₂. 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 B₁, B₂ G₁ and G₂ 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 B₁ and B₂ but 0.444 for AFs G₁ and G₂.

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 B₁ 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 B₁ 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 B₁ and B₂ residues were estimated in fresh liver samples 5.811 ± 1.049 for each, while AFs G₁, M₁ and M₂ were detected in one sample thus they were not included in the analysis, followed by 4.815 ± 2.185 for AF B₁ in small intestine then 4.096 ± 2.905 for AF B₁ in lung samples finally 1.573 ± 0.635 for AF B₁ in tongue.

In case of cooked samples, the highest mean value of AF residues, AF B₁ 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 M₁ and M₂ 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 public 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 detected, 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 be an effective means of attaining meat free of AF residues.

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