Study on Aflatoxin Residues in some Meat Products and their Control by Probiotics

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A B S T R A C T

Mould not only causes deterioration of food and feed, but also adversely affects the health of man and animals since it is capable of producing toxic metabolites known as mycotoxins causing food poisoning and liver cancer. Thus, creating awareness about aflatoxin residues and their control in food is of great importance for public health. From this point of view, this study was designed to evaluate total aflatoxin residues and their control by probiotics. A total of 100 random samples of meat products represented by beef burger, sausage, minced meat and luncheon (25 of each), were collected from different super markets in Cairo governorate. The obtained results revealed that the total aflatoxin residues were higher in luncheon (1.63±0.32 ppb) with the highest prevalence (88%). Also, the effect of probiotics on the reduction of aflatoxin residues in naturally contaminated minced meat sample was studied. Two probiotic strains (*Lactobacillus acidophilus* and *Bifidobacteriumlactis*) could be able to cause gradual reduction in total aflatoxin residues up to 88% and 98.3%, respectively of total aflatoxin residues within 8 days of experiment.

Keywords: Aflatoxin residues, AFT, AFB1, *Lactobacillus acidophilus*, *Bifidobacteriumlactis* and ELISA.

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1. INTRODUCTION

Meat products are considered favourable foods as they are easy to buy, fast to cook, delicious to eat, so they are the first choice for many people all over the world (Heinz and Hautzinger, 2007). On the other hand, meat products may be contaminated with moulds as they are widely distributed in nature and may contaminate meat and meat products through several ways. The environment inside slaughter houses and butcher-shops including walls, floors, utensils, hides and the intestinal contents of food animals, as well as tables, knives and refrigerators are considered as the main sources of fungal contamination of meat. Mycotoxinogenic moulds such as *Aspergillus*, *Fusarium* and *Penicillium* play undesirable role in the deterioration of the marketable quality and hygiene of food stuffs by synthesizing highly toxic metabolites known as mycotoxins. The occurrence of mycotoxigenic moulds in food is potentially dangerous for public health and also

constitutes a major economic problem (Dalie *et al*., 2010).

Aflatoxins are highly toxic fungal secondary metabolites that if ingested can cause a variety of adverse effects on both human and animal. Aflatoxins are carcinogenic compounds produced predominantly by certain species of Aspergillus, especially *A. flavus* and *A. parasiticus*. These fungi can grow on a wide variety of foods and feeds under favorable temperature and humidity. Contamination by aflatoxins can take place at any point along the food chain (Giray *et al*., 2007). Aflatoxins are common contaminants of foods, particularly in the staple diet of many countries, and they are categorized as class 1 A human carcinogen by International Agency for Research on Cancer (IARC, 2002). They have immunosuppressive, mutagenic, teratogenic and carcinogenic effects, especially on the liver. Food and feedstuff contaminated with aflatoxins (AFTs) is a serious health problem for man and animals, especially in developing countries Ghazvini *et al*. (2016).

A variety of physical, chemical and biological methods have been developed for decontamination and control of aflatoxins from contaminated goods and feeds (Morteza *et al*., 2013). The probiotic bacteria are living microorganisms which upon ingestion in certain numbers exert health benefits beyond inherent basic nutrition. The empirical use of microorganisms and/or their natural products for the preservation of foods (bio preservation) has been a common practice in the history of mankind (Ross *et al.,* 2002).

There is a great chance for reducing the presence of AFs in food products through the utilization of certain types of nonpathogenic bacteria such as the group of LAB. Also, utilization of LAB as a food supplement or probiotic products preventing the absorption of AFB1 in human and animal bodies as they have the ability for binding and isolating AFB1(Farber *et al*., 2000 and Hamidi *et al*.,2013).

Moreover, several studies have suggested that the antimutagenic and anticarcinogenic properties of probiotic bacteria can be attributed to their ability to non-covalently bind hazardous chemical compounds such as aflatoxins in the colon.

Lactic acid bacteria work as biological absorbents that prevent aflatoxins transfer to the intestinal tract of man and animals. *Lactobacilli* especially are relatively well studied and provide noticeable possibilities in binding of aflatoxin B_1 and M_1 in food (Sara *et al*. 2015).

This study was designed to determine the total aflatoxin residues in the examined meat products and to investigate the ability of *Lactobacillus acidophilus* and *Bifidobacteriumlactis* to reduce total aflatoxin residues in naturally contaminated minced meat sample.

2. MATERIALS AND METHODS

2.1. Collection of samples:

A total of 100 random samples of meat products represented by beef burger, sausage, minced meat and luncheon (25 of each), were collected from different super markets in Cairo governorate. Each sample weighed about 100 g and aseptically transferred without delay, in an insulated ice box to the laboratory and subjected to mycological examination and aflatoxin detection.

2.2. Estimation of total aflatoxins (Sahar et al., 2013)

The quantitative analysis of total aflatoxins was determined through a competetive direct enzyme linked immunosorbent assay (CD-ELISA) method. The method based on accurate monitoring of mycotoxins. The

veratox test kits (Neogen Crop., Lansing, MI. USK. Approved by the AOAC research institute (certificate No 950702) and the USDA-GIPSA (2008-011) were used. The analysis was done according to the manufacturer's instructions. Concentration of aflatoxins was calculated by log/log it software Awarness Technology Inc. (Anonymous, 2000 and Stoloff *et al*.,1999).

Calculation: For quantitative results, absorbance values obtained for the standards and the samples were divided by the absorbance value of the first standard (zero standard) and multiplied by 100 (percentage maximum absorbance). Therefore, the zero standard is thus made equal to 100% and the absorbance values are quoted in percentages. plotting the standard curve on the semi logarithmic graph paper, placing the value of standards on x-axis and corresponding absorbance value on Y-axis. The concentration of TAF.

Levels in the tested samples were estimated from the standard curve relation optical density versus TAF standards.

2.3. Preparation of LAB inoculums

Lactobacillus acidophilus was originally obtained from Ch. Hansen's Lab. (Denmark), and *Bifidobacteriumlactis* which obtained from Australian Research Center Australia, they were reactivated by three consecutive sub culturing on De Man Regosa & Sharp medium (MRS) broth and agar at 37 °C for 24 hrs. The suspensions were centrifuged at 1.700 X g for 15 minutes. The supernatant was discarded, and the bacterial pellets were washed twice with phosphate buffered saline (PBS; PH 7.3, 0.01 M) and the concentration of *Lactobacillus acidophilus* and *Bifidobacteriumlactis* was adjusted to obtain desired inoculums level 10^7cfu/ml (Maha *et al*., 2015)

2.4. Decontamination of total aflatoxins by LAB:

Fresh minced meat sample (200 gm) which contain known amount of TAF (4.212 ppb) that was estimated using ELISA was divided into two groups, each group inoculated with *Lactobacillus acidophilus* and *Bifidobacteriumlactis* in conc. of 10^7cfu/g, separately. Then samples were examined for total aflatoxin residues at zero time and every 48 hrs using ELISA.

3. RESULTS

Table (1): Statistical analytical results (ppb) and acceptability of total aflatoxin residual levels in examined meat products (n= 25 of each)

Meat products	Positive samples		Min	Max	Mean ± SE ppb	Pl*. ppb	Accepted Samples		Unaccepted samples	
	No	%					No	%	No	%
Burger	16	64	0.1	3.1	$0.73 \pm 0.15^{(a)}$	20	25	100	0	0
Sausage	20	80	0.1	2.5	$0.77 \pm 0.20^{(a)}$	20	25	100	0	0
Minced meat	18	72	0.1	2.5	$0.65 \pm 0.14^{(a)}$	20	25	100	0	0
Luncheon	22	88	0.2	6.0	$1.63 \pm 0.32^{(A)}$	20	25	100	0	0

There were highly significance differences (P>0.01) between capital and small letters within the same coloum N.B: Pl* according to FDA (2000) and FAO (2004).

Table (2): Effect of different probiotics on reduction percentage of total aflatoxins in experimentally contaminated minced meat sample:

Type of probiotic	Zero time		2nd day		4th day		6th day	
	Conc (pp)	Reduction %	Conc (ppb)	Reduction %	Conc (ppb)	Reduction %	Conc (ppb)	Reduction %
Group A	4.212	0	2.991	29	2.11	50	0.505	88
Group B	4.212	0	1.116	73.5	0.303	92.8	0.075	98.2

Group A: Samples treated with *Lactobacillus acidophilus*.
Group B: samples treated with *Bifidobacteriumlactis.*

4. DISCUSSION

4.1-Determination of aflatoxin residual levels in examined meat products

Mycotoxins are fungal secondary metabolites that if ingested can cause a variety of adverse effects on both human and animal. Aflatoxins are carcinogenic compounds produced predominantly by certain strains of *Aspergillus* genus. They have immunosuppressive, mutagenic, teratogenic and carcinogenic effects, especially on the liver Morteza *et al*. (2013). Aflatoxin can enter the food supply by direct contamination which resulted from mould growth on the food, or indirectly through the use of contaminated ingredients in processed food or by feeding mouldy feed to food producing animals. Indirect contamination of food may be a problem in some area of the world where food is more highly processed Bahagt *et al.* (1999). Table and Fig. (1) showed that AFT residual levels in all examined meat product samples were within the permissible level of total aflatoxin residues (20 ppb) which has been specified by FDA (2000) and FAO (2004). Burger, sausage and minced meat samples contained lower level of aflatoxin residues as compared with luncheon samples (there was a highly significant difference (P>0.01). Mean total aflatoxin residual level was0.73±0.15ppb in 16(64%) out of 25 examined burger samples. Nearly similar low aflatoxin B1 residual level in burger was recorded by El-Mossalami (2010) (0.41 ppb). Higher results were obtained by El-Shafei (2007) (40% were contaminated with 14.89 ppb), Abd-Elghany and Sallam (2015) who found that all 25 examined burger samples (100%) collected from Mansoura city were contaminated with (3.22 ppb) total aflatoxin (AFT), out of which, 40% were exceeded FAO, AFT permissible limit and Gehad *et al.* (2017) who found total aflatoxin average ±SE, 4.17± 0.66).

In this respect. Zohri *et al.* (2014) could detect 6 samples (30%) contaminated with AFT; one sample (6%) was contained >100 ppb and the remained 5 samples contained <50 ppb. Moreover, Maktabi *et al.* (2016) who found 3 (6.3%) of burger samples were contaminated with >1 µg/kg AFT.

Table (1) shwoed that the mean aflatoxin residual level in examined sausage samples was 0.77± 0.20in 20(80%) out of 25 examined sausage samples. Higher AFT residual levels were recorded by several investigators; Hassan and Ragheb (1996) {6 samples (15%) contained 1280 ppb, 1(2.5%) contained 160 ppb, 2(5%) contained 84 ppb and 3 samples (7.5%) contained 486 ppb residual AFT, Brr *et al.* (2004) (35-50 ppb for AFB1 in all samples, 17-22 ppb AFB2, 45-50 ppb AFG1and 20-22 AFG2). Lower prevalence with high residual levels were recorded by Dalia (2012) (55% with mean 15.22± 3.40 ppb AFT), Markov *et al*. (2013) (10% contained 3.0 ppb residual AFB1) and Maktabi *et al.* (2016) {2 samples (4.9%) were contaminated with >1 µg/kg AFT}. Lower prevalence of AFT was recorded by,Ismail *et al*. (2013) who could not detect AFB1 residues in examined sausage samples and Zohri *et al.* (2014) (10%).

Regarding AFT residual levels in examined minced meat samples (Table 1 and Fig.1), mean ppb was 0.65±0.14 where18 (72%) were positive. Higher results of AFT residual levels were recorded by El-Shafei (2007) (AFT detected with 8.52ppb in 20% of examined samples) and Mohammed (2015) (B1, B2, G1 and G2 were (3.62±0.88, 3.40± 0.82. 4.24 ± 0.85 and 2.83± 0.60), respectively. Lower prevalence of AFT residual levels was recorded by Hassan *et al.* (1997) (16.6%).

Results of AFT residual levels mentioned in Table and Fig. (1) revealed that mean AFT (ppb) in examined luncheon samples were 1.63±0.32. Luncheon samples contained the highest prevalence 22(88%) as compared with other products. Nearly similar results for AFT residual level was recorded by Mohamed *et al*. (2014) (1.1 ppb) in Mansoura city, in addition, all samples had a lower AFT permissible residual level by FDA (2012), while the prevalence of samples contained AFT obtained by the author was higher (25/100%) than that recorded in the present study. Higher AFT residual levels were reported by several investigators; Ismail *et al*. (2013) (AFB1, B2, G1 and G2 were 3.71± 1.35, 3.59± 1.12, 5.24± 1.12 and 6.77±1.49 ppb, respectively) and Gehad *et al.* (2017) (5.44± 0.39 ppb). Lower prevalence with high AFT residual level was recorded by Khater (2004) (examined luncheon samples were contained aflatoxin B1 minimum 1.3, maximum 24.5 and average ± SE, 10.4±5.1 ppb) and Dalia (2012) (25%, contained 3.92 ± 0.88 ppb for AFT).

The production of mycotoxins in meat and meat products can be fostered by the presence of oxygen, temperatures between 4 and 40 ºC, pH values between 2.0 and 8.0, a minimum aw of 0.80, and a maximum salt concentration of 14% Ostry and Ruprich (2001). It seems that there is no relationship between the presence of toxogenic strains of moulds and mycotoxin contamination of meat samples, as it is not cleared whether aflatoxin was produced during meat processing or it was present before as a residual level in muscles Ismail and Zaki (1999).

4-2- Effect of different probiotics on the reduction of total aflatoxins (AFT) in naturally contaminated minced meat:

Food and feedstuff contamination with aflatoxins (AFTs) is a serious health problem for humans and animals, especially in developing countries (Ghazvini *et al*., 2016 and Silvia, 2007) Vinderola and |Ritieni (2015) revealed that Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host. They commonly belong to the genera *Bifidobacterium* and *Lactobacillus*, as they are the most acceptances by the international scientific community joint FAO/WHO working group (FAO/WHO, 2002).

Table (2) explained using two groups of naturally contaminated minced meat samples with aflatoxins; the 1st group (A) was inoculated with 7 log_{10}cfu/g *Lactobacillus acidophilus* and the 2nd group (B) was inoculated with 7 log_{10}cfu/g *Bifidobacteriumlactis*.

The two groups were stored at 4°C and examined at zero time, 2, 4, 6 and 8 days of storage to determine the effect of used probiotics on the reduction of total aflatoxins. The obtained results revealed the following. At zero time, the concentrations of total aflatoxins remain constant with zero reduction % for both probiotics used in group A & B. At the 2nd day of storage, AFT was reduced by 29%, recorded 2.991 ppb. For group (A), while group (B) recorded 1.116 AFT concentration with 73.5% reduction rate. At the 4th day of storage, AFT concentration reduced to 2.11(50%) and 0.303 (92.8%) in group A and B, respectively. At 6th day of storage, AFT concentration recorded 0.505 with 88% reduction rate for group (A) and 0.075 (98.3%) for group (B). from the obtained results, it could be concluded that *Bifidobacteriumlactis* found to be more effective in elimination of AFT from contaminated minced meat than *Lactobacillus acidophilus.*

Similar results were recorded by several investigators; El-Nezami *et al.* (1998) who found that probiotics had the ability to bind with aflatoxins B1 and removed it, Haskard *et al*. (2017) found that probiotics strains including *L. rhamnosus* GG and *L. rhamnosus*LC-705 were the most efficient strains in removing AFB1, with removal rates of 78.9 % and 76.5%, respectively, Soher*et al*. (2009) (achieved 100% complete inhibition of fungal growth and aflatoxin production by using cell free supernatants(CFS) from several *Lactobacillus* species and she also reported that *L. acidophilus* recorded the highest inhibitory effect on the germination of *A. parasiticus*.

Maha *et al.* (2015) reported complete elimination of aflatoxin M1 by adding *L. acidophilus* and *B. lactis* to naturally contaminated milk with 50.2 ppt after 3 days of cold storage at refrigerator.

In a study carried out by Ghazvini *et al*. (2016) found that LAB was able to reduce total aflatoxins and B_1, B_2, G_1 and G_2 fractions by more than 99%. Moreover, LAB metabolites reduced the level of standard AFB_1, B_2, G_1 and G_2 from 88.8% to 99.8% ($p \leq 0.05$). Lower limit of aflatoxin B1 removal from contaminated food products than that of the present study was mentioned by Peltonen *et al.* (2001) observed that *Lactobacillus* and *Bifidobacteria* were able to remove 5.6% to 59.7% of AFB1, they added that, Bacterial binding of AFB_1 by some probiotic strains was rapid, and more than 50% of AFB_1 was bound throughout a 72-h incubation period and these findings further support the ability of specific strains of lactic acid bacteria to bind selected dietary contaminants.

Gratz *et al*. (2004) and Oatley *et al.* (2000) found that probiotics could bind significantly from 25% to 60% of added aflatoxins and made it unavailable for absorption in the intestinal tract. The authors suggested that there are reproducible strain differences in AFB1 binding capacity, Shahin (2007) observed that *L. lactis* and *S. thermophiles* could able to remove a greatest rate (71% to 86.7% and 66.5 % to 91.5 %) of AFB1, respectively where asnon viable cells of *L. lactis* could remove 100% of the toxin. Kabak *et al* (2006) (37%).Romina *et al*. (2011) reported that AFB1 binding ability of *Lactobacillus acidophilus* Po22 was 42.8 ± 1.7, *L. acidophilus*Po7 was 34.6 ± 1.6 and *L. acidophilus*24 was 32.6 ± 2.0., Haskard*et al.* (2017) (up to 71%).

Generally, the obtained results probiotics have the ability to make biological detoxification to aflatoxins as explained by Halasz *et al*. (2009) who revealed that biological detoxification of mycotoxins works mainly via two major processes, sorption and enzymatic degradation.

In fact, the potential presence of aflatoxins in animal diet is un avoidable, therefore a protection against aflatoxicosis is necessary, and the inclusion of microorganisms in the diet which able to remove AFB1 considered the most suitable alternative. The aflatoxin-microorganism mostly probiotics interaction is a fast, reversible and strain specific process, it is a physical adsorption to the cell wall of the microorganism Romina *et al*. (2011).

5. CONCLUSSION:

From the obtained results, one can conclud that there should be greater attention for consumption of mould contaminated meat products. A strict control against contamination of meat products with mould and aflatoxins.

LAB is naturally associated with many foods and are well recognized for their bio preservative properties. This study showed the ability of *Lactobacillus acidophilus* and *Bifidobacteriumlactis* to reduce aflatoxin levels through production of several low-molecular-weight antifungal metabolites, binding to the cell wall or combination of acidity and microbial competition. These antifungal LABS can be used in the food industry instead of chemical preservatives to produce organic foods. Furthermore, the excellent properties of LAB may preserve nutritional value of foods and delay spoilage. The future trends are to include beneficial probiotic microorganisms in a process of dietary detoxification of contaminated foods to constitute an approach for the decrease of the availability of aflatoxins in the human nutrition and animal feed. Due to their economic importance for the food industry and their health-related implications as probiotics safety assessment and risk analysis must be considered.

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