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Content and Health Risk Assessment of Total Aflatoxins in the Retailed Beef Luncheon, Sausage, and Pasterma in Zagazig City, Egypt

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Abstract

Meat products represent an important source of animal-derived protein. However, meat products are also implicated as a potential source of human exposure to some chemical residues such as aflatoxins (AFTs). The objectives of the present study were first to estimate the total AFTs content in the retailed meat products (beef luncheon, sausage, and pasterma) in Sharkia governorate, Egypt. Besides, dietary intake and potential health risks for the Egyptian population associated with the consumption of AFTs-contaminated meat products were further calculated. The obtained results revealed that pasterma had significantly the highest AFTs content, followed by luncheon, and sausage respectively. Calculation of the daily intakes of the total AFTs revealed that consumption of bovine meat products represents a potential source for dietary exposure to total AFTs. However, the calculation of the margin of exposure of total AFTs among the Egyptian population based on the average consumption of meat products revealed that such meat products might not possess a cancer risk among Egyptian adults and children. In general, excessive consumption of such meat products should be avoided for long-life protection against AFTs-related cancer risk.

KEYWORDS
Aflatoxin, Meat products, Health risk assessment, Egypt

INTRODUCTION

Meat products such as beef luncheon, sausage, and pasterma are regarded as important sources of animal-derived proteins and essential amino acids. Besides, meat products are very popular among children due to their specific aroma and flavor compared with red meat (Elhelaly *et al*., 2022; Morshdy *et al*., 2022). However, during the manufacturing process of such products, food additives and other non-meat ingredients such as spices, olives, etc are added. Such additives might facilitate contamination of the final products with some chemical contaminants such as mycotoxins, and heavy metals. In addition, inadequate preservation of the final meat products might lead to microbial contamination of the final products, particularly with different mold genera (Darwish *et al*., 2016).

Aflatoxins are the major mycotoxins that are produced by various mold species, such as *Aspergillus flavus* and *A. parasiticus* (Alcaide-Molina *et al*., 2009). Contamination of meat and meat products with AFTs might begin during the lifetime of the living animals via ingestion of contaminated animal feed. Besides, the growth of AFTs-producing molds might act as another contamination factor for meat products with AFTs, when the latter is produced. By consuming these contaminated products, humans are exposed to AFTs, which can have several toxicological effects (Aljazzar *et al*., 2021; Aljazzar *et al*., 2023). Immune suppression, mutagenicity, and carcinogenicity, especially hepatocellular carcinoma, are all side effects of AFTs (Abd-Elghany and Sallam 2015; Darwish *et al*., 2014). However, the available information about the occurrence of total AFTs in retailed meat products in Sharkia Governorate and their associated health risk assessment is very limited.

In light of the previously mentioned information, the current research set out to estimate the total aflatoxin levels in meat products sold in stores, such as beef luncheon, sausage, and pasterma. The Egyptian population's dietary intake and potential health hazards were also computed.

MATERIALS AND METHODS

All experiments were carried out according to the guidelines of Zagazig University, Egypt. No living animals were used in the current study. The used chemicals were of HPLC standard or the highest available quality including AFLASTANDARD, a total aflatoxin (B1+B2+G1+G2) purchased from R-Biopharm, Darmstadt, Germany.

Collection of Samples

Sixty samples including beef luncheon, sausage, and pasterma (n = 20/each, 100 g/ sample) were randomly collected from retail markets at Zagazig city, Sharkia governorate, Egypt from July to October 2022. Samples were separately packed and transferred cooled to the Central laboratory at the Faculty of Veterinary Medicine, Zagazig University, Egypt

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Estimation of total aflatoxins

Quantitative measurement of the total aflatoxins was completed using a Series-4EX Fluorimeter (VICAM, Milford, USA) and a few minor modifications to previous methods (El-Ghareeb *et al*., 2013; Abd-Elghany and Sallam, 2015). In summary, 100 mL of methanol: water (4:1 v/v) was used to blend 25 g of each sample for 3 min at high speed. The liquid was filtered using a 1.5 μm glass microfiber filter after being reduced with DDW four times. Following that, an AflaTest®-P affinity column was used to process 4 mL of the supernatant at a rate of 2 drops/s. Aflatoxin was extracted from the affinity column using HPLC grade methanol at a rate of 1 drop/s, and the complete sample eluate (1 mL) was collected in a glass cuvette (VICAM part # 34000). AflaTest® Developer 1.0 mL was added to the cuvette and carefully mixed with the eluate. The cuvette was then placed inside the adjusted fluorimeter. The AflaTest has a 0.1 (low detection) to 300 (high detection) ng/g measurement range. The limit of detection (LOD) for each of the examined arrays was 0.1 ng/g. The fluorimeter's excitation and emission wavelengths were fixed at 360 nm and 440 nm, respectively.

Estimated daily intake (EDI)

The US Environmental Protection Agency's (USEPA, 2010) Human Health Assessment Manual equation was used to calculate the estimated daily doses (EDI) of AFTs. Body weight (BW) was calculated as 70 kg for Egyptian adults and 30 kg for children, respectively, where C is the concentration of the food-contaminant tested (g/kg for AFTs), FIR is the meat ingestion rate in Egypt, which was fixed at 85.7 g/day for meat products, and BW is the meat ingestion rate (Abdallah *et al*., 2021). The AFTs' maximum permitted limits (MPLs) were also determined for samples using European Commission standards (EC, 2006). The benchmark MPL for AFTs in meat was set at 4 ng/g.

Health risk assessment

The daily intake of AFTs from the examined meat products was used to calculate the cancer risk among the Egyptian population using a margin of exposure (MOE) method (EFSA, 2005; FAO/WHO, 2006). To calculate the MOE, the benchmark dosage (BMDL10) that causes a 10% rise in the incidence of cancer in Fisher rats is contrasted with the average amount of the total consumption in people. The BMLD10 for AFTs was determined to be 250 ng/kg body weight/day. Benford *et al*. (2010) reported that the following algorithm was used to calculate MOE:
MOE = BMDL10/total consumption
MOE values below 10,000 indicate a severe health concern (EFSA, 2020)

Statistical analysis

The Tukey-Kramer HSD difference test was used to assess statistical significance after a one-way analysis of variance (ANOVA). To describe values, standard errors, and means were used. (SE). A statistical study was carried out utilizing (JMP). (SAS Institute, Cary, NC, USA). Significant data was defined as a p-value of 0.05 or less.

RESULTS AND DISCUSSION

According to the data in Fig. 1, 65%, 55%, and 25%, of the investigated samples of pasterma, luncheon, and sausage respectively, included AFTs. The highest total AFT residues were found in pasterma ($p < 0.05$), followed by luncheon and sausage. Given that the studied sausage, luncheon, and pasterma each had average concentrations of total AFTs (ng/g ww) of 3.59 ± 0.35, 2.99 ± 0.31, and 2.12 ± 0.39, respectively (Fig. 2). In pasterma, luncheon, and sausage, the percentages of samples that were above the MPL of AFTs (4.0 ng/g, EC, 2006) were 30%, 10%, and 5%, respectively (Fig. 3). Similarly, According to Pleadin *et al*. (2015), aflatoxin B1 was found in a variety of traditional meat products that were distributed throughout Croatian households and sold on the country's markets. During the research, traditional pork meat products like hams, dry-fermented sausages, bacon, and cooked sausage were tasted.

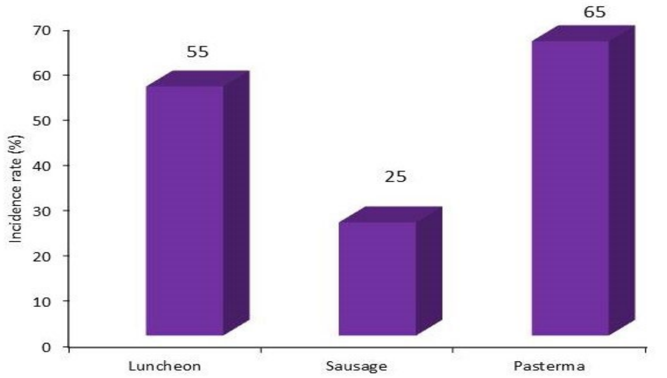

Fig. 1. Incidence of AFTs contamination in the examined beef luncheon, sausage and pasterma (n = 20/each).

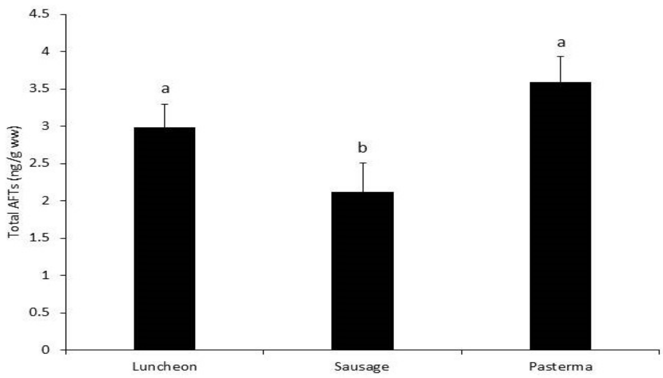

Fig. 2. Total AFTs content in the examined beef luncheon, sausage, and pasterma. Values represent means ± SE in the positive samples (n = 20 samples/each product). Columns carrying different letters (a, b) are significantly different at $p< 0.05$.

Mycotoxin concentrations were determined and confirmed using the validated immunoassay technique (ELISA) and high-performance liquid chromatography with fluorescence detection (HPLC-FLD), respectively. AFB1 concentrations in any meat product that was examined during this time were not appreciably greater ($p > 0.05$) than the detection limit established by the technique used. In Egypt, frozen chicken meat cuts and chicken giblets, including the liver and gizzards, were found to contain AFTs at similar levels by Darwish *et al*. (2016). Moreover, Cavus *et al*. (2018) assessed the mold and aflatoxin contamination of Turkish meat products (fresh meat, sausage, Turkish sucuk, and pasterma) as well as components (red paprika, black pepper, coriander, spice mix, and fenugreek powder) provided in Turkey. Out of a total of 55 identified *Aspergillus* and Penicillium species, *A. flavus* and A. niger were the two most prevalent mold species. Aflatoxin B1 was found in 50% (n = 12) and 65% (n = 13) of the meat products and additives, while mold amounts that exceeded

Turkish Food Codex standards were found in 25% and 43% of the sucuk and pasterma samples, respectively. However, one sample of red paprika contained amounts of AFB1 and total aflatoxin that exceeded those set by the Turkish Food Codex and the European Commission. According to Elzupir and Abdulkhair (2020), 37.5% of the processed meat product samples sold in Riyadh, Saudi Arabia, were contaminated, with average contamination levels of 6.4±12.58 μg/kg and concentrations reaching 52.93 μg/kg. 10% of contaminated samples had total AFs greater than the allowed Saudi limit of 20 g/kg, which was present in 4% of the samples that underwent contamination analysis. The most frequent toxins were AFB1 and AFG1, then AFB2; no samples contained AFG2. Aflatoxins were also estimated in imported meat products. AFTs may generally enter an animal's body through the consumption of contaminated feed, which could have negative impacts on the health and productivity of livestock around the world (Darwish *et al*., 2010; ; van der Fels-Klerx *et al*., 2018; Nishimwe *et al*., 2019).

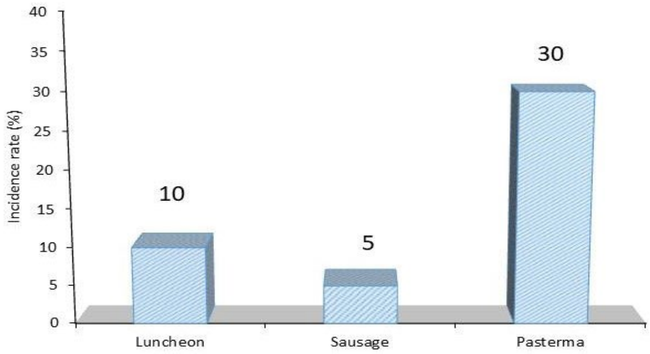

Fig. 3. Incidence (%) of samples exceeding MPL of AFTs in the examined beef luncheon, sausage, and pasterma (n = 20/each).

The obtained results in Fig. 4 indicated that EDI values for total AFTs via the average consumption of the bovine meat products were 2.59 (sausage), 6.4 (luncheon), and 7.69 (pasterma) in adults, while these values in children were 6.05, 9.96, and 11.97 ng/kg/BW, respectively. Consumption of such products represents an alarming source of dietary exposure to total AFTs among Egyptian consumers, particularly children.

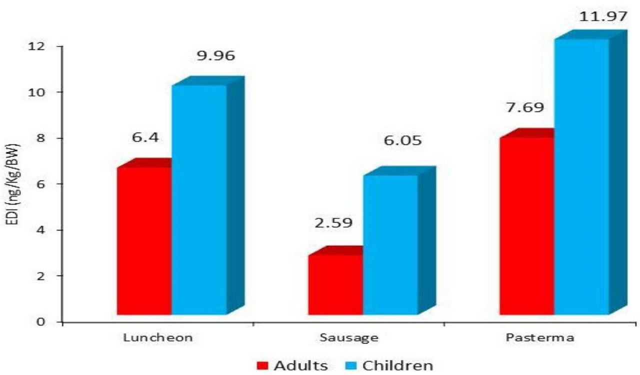

Fig. 4. EDI values (ng/Kg/BW) of the total AFTs among Egyptian adults and children due to consumption of beef sausage, luncheon, and Pasterma.

As a mutagen and a carcinogen, AFTB1 is classified (EFSA, 2007). Particularly in those with hepatitis C or B viruses, ingesting even low quantities of AFTs, as little as 1ng/kg/day, may cause liver cancer (American Cancer Society, 2011). Estimating the cancer risk for AFTs among Egyptians was done using the MOE technique (EFSA, 2005; FAO/WHO, 2006). MOE was regarded as the most precise and informative method to evaluate the cancer risk for AFTs. Adults' MOE values for the typical intake of AFTs varied from 32.47 for pasterma to 39.007 for luncheon to 96.03 for sausage, while children's MOE values were 20.87 for pasterma to 25.08 for luncheon to 41.03 for sausage (Fig. 5). These readings fell well below the established EFSA limit of 10,000 (EFSA, 2020), suggesting that eating such meat products might not increase your risk of developing cancer. However, excessive use of these products should be avoided, especially among children, to protect a long life. Likely, Elzupir and Abdulkhair (2020) stated that among the Saudi population, the MOE values for the total AFTs in processed beef meat and poultry meat products were 175 and 311, respectively.

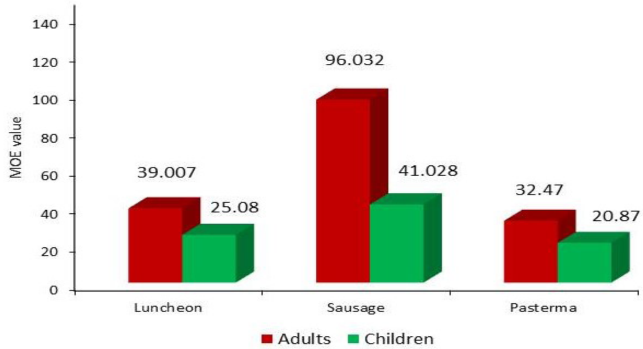

Fig. 5. MOE values calculated based on the average intake of the total AFTs among Egyptian adults and children due to consumption of beef sausage, luncheon, and Pasterma.

CONCLUSION

The current study's findings showed that retail meat products have AFT residues found in them. The highest residues for all AFTs were found at Pasterma. It was determined through risk analysis that eating such products might not be harmful to one's health. For the ongoing monitoring of total AFTs in meat products sold in Egypt's retail markets, additional investigations are still required. Thus, it is strongly advised to cut back on the daily consumption of meat products, especially among youngsters, to lower the chance of developing cancer and living longer.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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