



## Evaluating Probiotic Efficacy Against Mycotoxins Threat in Semi-Dry Fermented Beef Sausage

Nady Khairy Elbarbary<sup>1\*</sup>, Ashraf Abd El-malek<sup>2,3</sup>, Wageh Sobhy Darwish<sup>4</sup>, Sohaila Fathi El-Hawary<sup>5</sup>, Neveen M. Abdelmotilib<sup>6</sup>, Marwa A. Ali<sup>7</sup> and Mohamed K. Dandrawy<sup>8</sup>

<sup>1</sup>Food Hygiene and Control Department, Faculty of Veterinary Medicine, Aswan University, Aswan 81528, Egypt.

<sup>2</sup>Food Hygiene, Safety, and Technology Department, Faculty of Veterinary Medicine, Assiut University, Assiut 71526, Egypt.

<sup>3</sup>School of Veterinary Medicine, Badr University, Assiut, Egypt.

<sup>4</sup>Food Hygiene, Safety, and Technology Department, Faculty of Veterinary Medicine, Zagazig University, Zagazig 44519, Egypt.

<sup>5</sup>Biology Department, Collage of Science, Jazan University, P.O. Box 114, Jazan 45142, Kingdom of Saudi Arabia.

<sup>6</sup>Food Technology Department, Arid Lands Cultivation Research Institute (ALCRI), City of Scientific Research and Technological Applications (SRTA-CITY), New Borg El-Arab City 21934, Egypt.

<sup>7</sup>Microbiologist, El Fayoum Laboratory for Microbiology and Immunology Analysis, El Fayoum 63511, Egypt.

<sup>8</sup>Food Hygiene and Control Department, Faculty of Veterinary Medicine, South Valley University, Qena, 83522, Egypt.

### Abstract

**P**ROBIOTIC consumption is recognized as being generally safe and correlates with multiple and valuable health benefits. Food exposure to mycotoxins is a major concern for public health officials and regulatory authorities globally. Aflatoxins (AFLs) and ochratoxin A (OTA) contamination of meat products can happen anywhere along the production process, from farm to fork. The purpose of this study is to determine the concentration of AFLs and OTA residues in some beef products and evaluate the effects of different probiotics (*Lactobacillus acidophilus*, *Bifidobacterium lactis* and *Saccharomyces cerevisiae*) on AFLs and OTA in semi-dry fermented beef sausage that has been contaminated in an experiment stored for seven days. The study found that the AFLs were present in 86.7%, 60%, 80%, 70%, 76.7%, and 70% of the meat products under investigation, while the OTA residues were present in 83.3%, 56.7%, 80%, 73.3%, 63.3%, and 76.7% of the burger, minced beef, luncheon, basterma, kofta, and sausage, respectively. Burger ( $13.89 \pm 2.62$  ppb) and sausage ( $12.67 \pm 2.37$  ppb) had the greatest AFLs residues (ppb), followed by kofta ( $11.38 \pm 2.15$  ppb) and luncheon ( $11.26 \pm 2.72$  ppb). Basterma ( $3.31 \pm 1.85$  ppb) and minced meat ( $5.47 \pm 1.55$  ppb) had the lowest values. Luncheon had the greatest OTA residues ( $2.76 \pm 0.43$  ppb), followed by burger ( $2.64 \pm 0.14$  ppb), sausage ( $2.32 \pm 0.57$  ppb), and kofta ( $1.78 \pm 0.74$  ppb), while basterma ( $1.23 \pm 0.65$  ppb) and minced beef ( $1.56 \pm 0.12$  ppb) had the lowest concentrations. The findings reveal that the levels of AFLs in some examined samples exceeded the legal limits ( $< 20$  ppb), while the levels of OTA were within the acceptable range ( $< 5$  ppb). The data shows a positive association between the use of probiotics and the reduction of AFLs and OTA in all samples studied. The results indicate that probiotics such as *Lactobacillus acidophilus*, *Bifidobacterium lactis* and *Saccharomyces cerevisiae* can potentially serve as decontaminants in the food industry as well as can replace chemical preservatives in producing organic foods and reduce the levels of mycotoxins in beef products intended for human consumption.

**Keywords:** Aflatoxins, Detoxification, Meat Products, Probiotics, Ochratoxin.

### Introduction

Meat products are widely favored foods due to their accessibility, preparation, and palatability, which contribute to their status as preferred options among numerous individuals worldwide [1]. Mycotoxins are harmful secondary metabolites created by fungi when they colonize food sources. Mycotoxins

contaminate meat products at various stages of production, either through the direct introduction of contaminated spices or indirectly via the ingestion of grains and feedstuffs contaminated with mycotoxins by animals. The contamination then spreads to consumers via meat, milk, and their products, resulting in carry-over effects [2]. These are strong

\*Corresponding author: Nady Kh. Elbarbary, E-mail: nadykhairy@vet.aswu.edu.eg, Tel.:00201003495017

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toxins that have serious health repercussions for humans, including mutagenicity, teratogenicity, and carcinogenesis [3]. Pandey *et al.* [4] identify taxa of *Aspergillus*, *Fusarium*, *Penicillium*, and *Alternaria* as the principal toxin-producing molds. Among the most dangerous mycotoxins, aflatoxins (AFLs) and ochratoxin A (OTA) are still a global concern that seriously impairs both human and animal health [5].

*Aspergillus flavus* and *Aspergillus parasiticus* primarily produce more than one aflatoxin, a highly toxic mycotoxin, globally [4]. The major categories are B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, and G<sub>2</sub> [6]. Regarding ochratoxins, food commodities contain three altered forms of them: OTA, OTB, and OTC. Various species, including *Penicillium verrucosum* and *Aspergillus*, synthesize OTA, the most widely detected mycotoxin in food products [4]. Because mycotoxins are resistant to enzymatic degradation and autolysis in the gastrointestinal tract (GIT) of animals, they are present in the meat. Cooking or processing food does not break down most mycotoxins, as they can withstand high temperatures [7]. Consumption of mycotoxin-contaminated food items harms human and livestock health, reducing food commodity marketability and raising food safety issues [8]. A mycotoxin can cause acute or chronic toxicity, including hepatotoxicity, cytotoxicity, teratogenicity, neurotoxicity, mutagenicity, and carcinogenicity, which is known as mycotoxicosis. Some mycotoxins interfere with nucleic acids at the cellular level, inhibiting DNA and RNA synthesis [9].

Probiotics are defined as "living microorganisms that, when given in sufficient quantities, improve the host's health" by the World Health Organization [10]. Lactic acid bacteria (LAB), notably *Lactobacillus* and *Bifidobacterium*, are the most often used forms of conventional probiotics available nowadays. These bacteria help treat various intestinal disorders [11]. Probiotic bacteria and yeast help to lower the adhesion of bacteria to the intestinal mucosa, therefore lowering bacterial toxins and producing antibacterial substances including bacteriocins and antibiotics [12]. Furthermore, Kerry *et al.* [13] have established the crucial role of the aforementioned probiotic metabolites in promoting gastrointestinal health and preserving intestinal homeostasis. It is critical to screen the occurrence of mycotoxins in animal feeds and livestock products to safeguard both humans and animals from these potentially harmful substances.

The purpose of this research is to determine the concentration of AFLs and OTA residues in some beef products and evaluate the effects of different probiotics, such as *Lactobacillus acidophilus* (*L. acidophilus*), *Bifidobacterium lactis* (*B. lactis*), and *Saccharomyces cerevisiae* (*S. cerevisiae*), on AFLs and OTA in semi-dry fermented beef sausage that has been contaminated in an experiment stored for seven days.

## **Material and Methods**

### *Samples collection*

A total of 180 beef products—burger, sausage, minced, luncheon, kofta, and basterma (30 of each)—were collected in January 2024 from supermarkets in Aswan, Egypt. Each sample, about 100 g, was transported to the laboratory in an icebox and stored in its original packages at 4 °C until analysis.

### *Estimation of the AFLs and OTA levels*

The analysis of AFLs according to Najmus *et al.* [14] and OTA according to Baydar *et al.* [15] was conducted at the Department of Food Hygiene Laboratory, Faculty of Veterinary Medicine, Aswan University, using a competitive direct enzyme-linked immunosorbent assay (CD-ELISA). The Veratox test kits (RIDASCREEN® Aflatoxin Total, Art. No.: R4701, and RIDASCREEN® Ochratoxin A 30/15, Art. No. R1312) from AG, Darmstadt, Germany, were employed. Following the manufacturer's guidelines, we analyzed the mycotoxins after pulverizing ten grams of each sample and extracting them with 50 ml of 70% methanol. An ELISA reader (absorbance microplate reader, model: ELX 808 IU, BIO-ITK, Instrument, INC, USA) was used to measure the absorbance at 450 nm. The percentage of maximal absorbance was calculated by dividing the absorbance values of the standard and samples by the absorbance value of the first standard (zero standard) and multiplying the result by 100. Consequently, the absorbance values are expressed in percentages, and the zero standard is equated to 100%. The optical density versus AFLs and OTA standards concentration standard curve was used to estimate the level of AFLs and OTA levels in the tested samples.

### *Preparation of the probiotic inocula*

*L. acidophilus* was supplied from Ch. Hansen's Lab. (Denmark), *B. lactis* from the Australian Research Centre, and *S. cerevisiae* from baker's

shops as baker's yeast. Triplicate subcultures on De Man, Rogosa, and Sharpe (MRS) broth and agar at 37 °C for 24 h were used to reactivate *L. acidophilus* and *B. lactis*. The suspensions were centrifuged at 1,700 x g for 15 min. The bacterial pellets were rinsed twice with phosphate-buffered saline (PBS; pH 7.3, 0.01 M), and the concentration was adjusted to 10<sup>7</sup> CFU/mL, equivalent to a 0.5 McFarland standard, after the supernatant was removed [1,16]. *S. cerevisiae* was employed at concentrations of 1% (10<sup>5</sup> CFU/mL) and 2% (10<sup>8</sup> CFU/mL) [7].

#### *Preparation of semi-dry fermented beef sausage*

The recipe for semi-dry fermented sausage was made using the method of Emara et al. [17] 80% beef topside, 15% beef fat, 2.0% sodium chloride, 0.02% sodium nitrite, 0.05% ascorbic acid, 1% lactose, 0.50% glucose, and 0.05% spice were mixed under good manufacturing practices. The dried non-meat ingredients were combined with the ground beef and fat in a Seydelmann spiral mixer (Urgstallstraße, Germany).

#### *Decontamination of AFLs and OTA by probiotics*

OTA and AFLs standards were added to the semi-dry fermented sausage mixture at a concentration of 100 µL/100 g (above the standard concentration of the regulatory authority's < 20 ppb). The sausage was divided into four equal groups; G1, G2, G3, and G4 were inoculated separately with *L. acidophilus* (G1) and *B. lactis* (G2) at 10<sup>7</sup> CFU/g and to each of them *S. cerevisiae* at 1% (G3) and 2% (G4), v/v, respectively. The sausage mix was stuffed into 30 mm cellulose casings (500 g each) with the Handtmann VF 628 vacuum filler (Baden-Württemberg, Germany) and kept in a ripening chamber at 20 °C and 70% relative humidity for 4 days to reach a pH of 5.20. After ripening, the sausages were stored at 4 °C for 7 days. The efficacy of probiotics in the amelioration of AFLs and OTA in semi-dry fermented sausage was experimentally evaluated by repeating the experiment three times with three replicates at independent time points (0, 2, 5, and 7 days).

#### *Statistical analysis*

Data with a significance level of  $p < 0.05$  were reported as the mean of three replicates. Standard errors were statistically assessed using the analysis of variance technique (ANOVA) in SAS [18].

## **Results**

### *Estimation of the AFLs and OTA levels*

Figure 1 shows that 86.7%, 60%, 80%, 70%, 76.7%, and 70% of the beef samples tested (burger, minced beef, luncheon, basterma, kofta, and sausage) had AFLs. On the other hand, 83.3%, 56.7%, 80%, 73.3%, 63.3%, and 76.7% of the burger, minced beef, luncheon, basterma, kofta, and sausage samples were contaminated with OTA. The most products have AFLs residue were burgers and sausages, with average levels of  $13.89 \pm 2.62$  ppb and  $12.67 \pm 2.37$  ppb, respectively, followed by kofta and luncheon, with average levels of  $11.38 \pm 2.15$  ppb and  $11.26 \pm 2.72$  ppb. Finally, basterma and minced beef had the lowest levels, with  $3.31 \pm 1.85$  ppb and  $5.47 \pm 1.55$  ppb, respectively. There is statistically considerable variation in the AFLs residue between the examined products ( $p < 0.001$ ). The authority (FAO, 2004) approved the aflatoxin residues in minced beef and basterma samples, while burger, kofta, luncheon, and sausage samples, at 20%, 13.3%, 26.7%, and 26.7%, respectively, exceeded the authorized and controlling limits (< 20 ppb) for beef products (Table 1).

Concerning the incidence of OTA in the inspected samples (Table 2), the highest OTA residues were found in luncheon ( $2.76 \pm 0.43$  ppb), followed by burger ( $2.64 \pm 0.14$  ppb), sausage ( $2.32 \pm 0.57$  ppb), and kofta ( $1.78 \pm 0.74$  ppb) while the lowest concentration recorded in basterma ( $1.23 \pm 0.65$  ppb) and minced beef ( $1.56 \pm 0.12$  ppb) with a significant variation between the examined samples ( $p < 0.05$ ). Concerning the recommendations of authorities (FAO, 2004 and WHO, 2002), no samples above the legal and guiding limits (< 5 ppb) for beef products.

#### *Decontamination of AFLs and OTA by probiotics*

Table 3 and Fig. 2 show the effect of different probiotics on AFLs concentration (ppb) in experimentally contaminated semi-dry fermented beef sausage samples; the findings demonstrate a positive association between the usages of probiotics and the reduction percent of AFLs in all samples analyzed. At zero time, the total AFL levels were 4.632, 4.597, 4.613, and 4.588 ppb for G1, G2, G3, and G4, respectively, with no reduction in percentage for either probiotic used in any of the groups. On the 7<sup>th</sup> day of cold storage, G1 recorded the best results; AFLs reduced by 35.8% on the 2<sup>nd</sup> day, 73% on the 5<sup>th</sup> day, and reached the maximum (98.2%) on the 7<sup>th</sup> day, with recorded concentrations of 2.976, 1.264, and 0.085 ppb, respectively. G2 recorded AFLs concentrations of 3.172, 1.422, and 0.774 ppb with reduction rates of 31%, 69%, and 83.2% on the 2<sup>nd</sup>, 5<sup>th</sup>, and 7<sup>th</sup> day, respectively. G3 recorded the lowest

AFLs reduction (3.637, 1.638, and 1.004 ppb) with reduction rates of 21.2%, 64.5%, and 78.2% on the 2<sup>nd</sup>, 5<sup>th</sup>, and 7<sup>th</sup> day, respectively. On the 2<sup>nd</sup>, 5<sup>th</sup>, and 7<sup>th</sup> day, G4 reported AFLs concentrations of 3.287, 1.376, and 0.867 ppb, with reduction rates of 28.4%, 70%, and 81.1%, respectively. The discrepancy in the meat products sample reviewed was exceedingly substantial ( $p < 0.05$ ) except for G2 and G4.

According to Table 4 and Fig. 3, G1 has the highest reduction rate (16.5%, 30.7%, and 69.8%) and the highest OTA concentration (1.332, 1.106, and 0.482 ppb) on 2<sup>nd</sup>, 5<sup>th</sup>, and 7<sup>th</sup> days, respectively. G4 is next, with a reduction rate of 14.8%, 27%, and 58.3% and OTA concentrations of 1.38, 1.18, and 0.679 ppb on the 2<sup>nd</sup>, 5<sup>th</sup>, and 7<sup>th</sup> day, respectively; and G2 had OTA concentrations of 1.482, 1.268, and 0.854 ppb on the 2<sup>nd</sup>, 5<sup>th</sup>, and 7<sup>th</sup> day, respectively, and a reduction rate of 10.1%, 23%, and 48.2%. Additionally, G3 recorded the lowest reduction rate (7.8%, 17.1%, and 41.5%) and concentrations of 1.533, 1.378, and 0.973 ppb, respectively, on the 2<sup>nd</sup>, 5<sup>th</sup>, and 7<sup>th</sup> day. But at zero time, all treated groups have zero reduction %. Moreover, the reduction of OTA in examined samples is significantly different ( $p < 0.05$ ).

## **Discussion**

Meat products are regarded as the major desired and promising food due to their high nutritive value, which is attributed to the substantial amount of vital amino acids, minerals, fats, and vitamins [19]. The contamination of beef products with mycotoxins poses a public health risk to customers. Many investigations found that mycotoxins have mutagenic, carcinogenic, nephrotoxic, hepatotoxic, teratogenic, immunosuppressive, and embryotoxic effects [20]. The data in Figure 1 show that burger and luncheon samples had the highest occurrence rates (86.7% and 80%, respectively), whereas minced beef samples had the lowest (60%). These results were similar to those of Karmi [7], who found AFLs residues in 80% of basterma, 96% of luncheon, 92% of minced beef, 76% of kofta, and 88% of minced beef. These findings differed from those of Ibrahim *et al.* [1], who found 64%, 72%, 88%, and 80% for burger, minced beef, luncheon, and sausage, respectively. Furthermore, Algammal *et al.* [19] identified AFL residues in 6% of basterma samples, but they were unable to detect any residues in minced beef and sausage samples. Morshdy *et al.* [21] found that AFTs were present in 65%, 55%, and 25% of the sausage, basterma, and luncheon samples that were

analyzed. Elbarbary *et al.* [22] found that all of the samples that were analyzed (100%) had AFL residues.

Aflatoxins are the most dangerous mycotoxins that infect the human diet. Molds frequently contaminate preserved meat products, including sausage, basterma, and burgers, which become active due to the extended maturation process and subsequent production of mycotoxins [19]. The current investigation presents the AFL residues in Table 1. The data showed a substantial difference ( $p < 0.001$ ) among the studied samples, except for burgers and sausages showing no significant variance. AFLs concentration was highest in the burger ( $13.89 \pm 2.62$  ppb) and sausage ( $12.67 \pm 2.37$  ppb) samples, while basterma ( $3.31 \pm 1.85$  ppb) and minced meat ( $5.47 \pm 1.55$  ppb) had the lowest concentrations. The amount of AFLs residue found in minced beef and basterma samples were within acceptable limits, but the amounts found in the luncheon, burger, and kofta samples were higher than the international limit of FAO [23] for beef products ( $< 20$  ppb) by 26.7%, 20%, and 13.3%, respectively. Elbarbary *et al.* [22] reported lower results, indicating that the AFL concentration (ppb) in burgers, minced beef, luncheon, kofta, and sausage was  $5.4 \pm 0.13$ ,  $3.9 \pm 0.28$ ,  $5.14 \pm 0.18$ ,  $5.68 \pm 0.2$ , and  $9.85 \pm 0.64$ , respectively. These readings were significantly lower than the established limit of FAO [23], except for 40% of sausage samples. According to Morshdy *et al.* [21], basterma had the greatest total AFLs residue ( $3.59 \pm 0.35$  ppb), followed by luncheon ( $2.99 \pm 0.31$  ppb) and sausage ( $2.12 \pm 0.39$  ppb). Additionally, 30%, 10%, and 5% of basterma, luncheon, and sausage exceeded the legal limit for AFLs. Elzupir and Abdulkhair [24] reported that 37.5% of processed meat product samples were polluted, with levels up to 52.93 ppb. Additionally, 10% of contaminated samples had total AFs above the Saudi Arabia limit (20 ppb). Additionally, Karmi [7] reported that the concentration of AFLs residue in the inspected samples was 2.6 ppb in basterma, 2.7 ppb in a burger, 2.3 ppb in luncheon, 2.5 ppb in minced meat, and 2.7 ppb in kofta. All of the examined samples were within the international permissible limits. Additionally, Ibrahim *et al.*<sup>1</sup> found that the tested samples did not surpass the allowable levels for burger, minced beef, luncheon, and sausage, with respective results of 0.73, 0.65, 1.63, and 0.77 ppb. A study by Kademi *et al.* [25] reports that mycotoxigenic fungi can contaminate meat products and make AFLs, OTA, and other

mycotoxins at different stages of the manufacturing process, from polluting food for livestock on the farm to eating the finished product at the table. Yang et al. [26] believe that food additives, particularly spices, are a major source of mycotoxin contamination during meat processing.

Ochratoxin A is a secondary metabolite that poses significant health hazards to both humans and animals. It is predominantly produced by *Aspergillus* spp. when the environmental and storage conditions are conducive to their growth and multiplication [27]. The majority of mycotoxins are heat-tolerant, so they are unaffected by cooking or processing [7]. According to the results in Fig. 1 and Table 2, no samples were above the legal and regulatory limitations ( $> 5$  ppb) for meat products [23,28]. Furthermore, OTA residual levels in burger, luncheon, and sausage samples differ considerably from minced beef, basterma, and kofta ( $p < 0.05$ ). The results reported by Karmi [7] were nearly identical, with OTA residues present in 92%, 96%, 80%, 72%, and 88% of basterma, burger, luncheon, minced beef, and kofta samples, respectively. The concentrations (ppb) were  $2.5 \pm 0.15$ ,  $1.04 \pm 0.14$ ,  $1.4 \pm 0.16$ ,  $1.03 \pm 0.14$ , and  $1.04 \pm 0.13$ , on average. Abd-Elghany and Sallam [29] found OTA in 67% to 100% of the analyzed samples, with concentrations of 7.8 ppb in sausage, 5.23 ppb in luncheon, and 4.55 ppb in burgers. Zadavec et al. [30] found OTA contamination in 14% of samples, with concentrations reaching 6.86 ppb in beef products. In the sausage samples that were examined, Algammal et al. [19] detected OTA residual in only 10% of the samples, with a mean value of  $10 \pm 2.9$  ppb. The examination of the minced meat and basterma samples yielded negative results for OTA. Moreover, Ulusoy et al. [2] failed to discover OTA residues in the analyzed samples. These fluctuations were attributed to changes in the number and types of additives used in meat product manufacture, changes in temperature and time exposure, and sanitary procedures utilized during processing. Moreover, the cattle's feed may have contaminated the meat with mycotoxins [22].

Food decontamination frequently employs physicochemical technologies, but these technologies require specific conditions that are often unattainable in numerous industrial sectors. Currently, probiotic strains and their enzymes perform biological detoxification, a promising technique for reducing the risk associated with the occurrence of xenobiotics in meals. The findings of numerous studies have

demonstrated that probiotics are an efficient, viable, and cost-effective method for preventing xenobiotic-induced dysbiosis and mitigating the adverse effects of these substances [30].

It was found that the amount of AFLs found in experimentally contaminated semi-dry fermented sausage samples dropped significantly ( $p < 0.05$ ) after different probiotics were added along with standard AFLs (Table 3 and Fig. 2). The groups treated with *L. acidophilus* (G1) showed a high reduction rate, which ranged from 35.8% on the 2<sup>nd</sup> day to 73% on the 4<sup>th</sup> day. This rate reached an optimal reduction of 98.2% on the 7<sup>th</sup> day of cold storage, accompanied by a decrease in concentration from 2.976 ppb on the 2<sup>nd</sup> day to 0.085 ppb on the 7<sup>th</sup> day. The impact of *B. lactis* (G2) and *S. cerevisiae* 2% (G4), on the reduction rate of the inoculated samples did not differ. All samples examined by the time show a good association between the consumption of various probiotics and their impact on experimentally contaminated semi-dry fermented sausage samples using standard OTA (Table 4 and Fig. 3). The results confirmed that the use of *L. acidophilus* (G1) and *S. cerevisiae* at 2% (G4) achieved the highest reduction rate (69.8% and 58.3%), followed by *B. lactis* treatment at 48.2%. Moreover, there is a significant variance ( $p < 0.05$ ) in the reduction of OTA in the studied samples.

*Lactobacillus acidophilus* and *Bifidobacterium lactis*'s potential mechanisms of detoxification are therefore linked to their ability to bind the toxic compounds due to the presence of peptidoglycan and polysaccharides in the cell wall. Reactive functional groups and compounds present in the cell wall, such as proteins, peptidoglycan, and polysaccharides; 1,3- $\beta$ -glucan for the yeast cell wall, are recognized to be responsible for probiotic binding capacity. The differences between the strains to toxin absorption and binding are probably due to the diversity in cell wall structures and bacterial cell membranes [31]. To sum up, the two hypotheses are attributed to the probiotic detoxification action. The first mechanism consists of the physical connection between the probiotic and contaminant. The second is when probiotics and strains can mitigate the carcinogenic danger through their metabolism. The cell wall of probiotics is primarily composed of peptidoglycan found in glycan chains consisting of alternating N-acetylglucosamine and N-2 acetylmuramic acid, linked by  $\beta$ -1,4 bond [32].

Probiotics and yeasts reduce the quantity of bacterial toxins by preventing pathogens from adhering to the intestinal epithelium while increasing the production of vitamins and antibacterial chemicals such as antibiotics and bacteriocins. Furthermore, probiotic metabolites play an important role in maintaining intestinal homeostasis and improving gastrointestinal health [13] and have an important function in modulating the host immune system [33].

Several studies came to the same conclusions. For example, Ibrahim *et al.* [1] tested two types of probiotics and found that they could reduce AFL residues by up to 88% and 98.3%, respectively, over 8 days. Haskard *et al.* [34] demonstrated that probiotic strains could potentially eliminate AFB1, achieving clearance rates of 78.9% and 76.5%, respectively. Additionally, Karmi [7] concluded that probiotics significantly lower AFLs and OTA in meat products. Researchers also discovered that *L. acidophilus* reduced AFLs and OTA in an experimentally spiked burger by 71.1% and 97.2%, respectively, while *S. cerevisiae* reduced them to 96% and 61%, respectively. The results show that probiotics may be able to biologically detoxify AFLs and OTA produced by fungi in several ways of action (decreasing intestinal pH, lowering colonization and multiplication of pathogens, metabolites, boosting the host immune response, bind toxins) [31]. This is in line with what Maha *et al.* [16] found, which was that probiotics could completely remove AFLs, and what Ghazvini *et al.* [35] found, which was that probiotics could lower AFLs by more than 99%. It is imperative to safeguard against mycotoxicosis, as the potential occurrence of AFLs and OTA in the diet is inevitable. The most appropriate alternative is the inclusion of microorganisms in the diet that are capable of removing AFLs and OTA. The US Food and Drug Administration (FDA) recently established

a regulatory framework for the use of "live biotherapeutic products" (LBP) in clinical applications, classifying them as biological products intended to prevent and cure mycotoxicosis [36].

### **Conclusion**

Some meat products in this research, subjected to varying degrees of AFLs and OTA residues, did not meet the regulatory limits. The results indicate that probiotics such as *L. acidophilus*, *B. lactis* and *S. cerevisiae* can potentially serve as decontaminants in the food industry as well as can replace chemical preservatives in producing organic foods and reduce the levels of mycotoxins in beef products intended for human consumption. Proper hygiene measures must be taken during the preparation and storage of processed meat; further research is also necessary to corroborate the findings regarding the utilization of probiotics.

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### **Declaration of Conflict of Interest**

No conflict of interest

### **Ethical of approval**

All techniques employed in this study were directed in compliance with applicable standards and regulations. The agreement was acquired from the Aswan Research Ethics Committee, Aswan University, Faculty of Veterinary Medicine (No. 02-11-2023).

**TABLE 1. Total AFL residues (ppb) in examined beef products and their acceptability\* (n=30 of each)**

Beef products	Min	Max	Mean±SE	Acceptable sample		Unacceptable sample	
				No	%	No	%
<b>Burger</b>	3.63	22.84	13.89±2.62 <sup>a</sup>	24	80	6	20
<b>Minced beef</b>	2.49	8.73	5.47 ±1.55 <sup>c</sup>	30	100	0	0
<b>Luncheon</b>	3.93	24.23	11.26 ±2.72 <sup>b</sup>	22	73.3	8	26.7
<b>Basterma</b>	1.38	5.62	3.31 ±1.85 <sup>d</sup>	30	100	0	0
<b>Kofta</b>	3.88	21.21	11.38 ±2.15 <sup>b</sup>	26	86.7	4	13.3
<b>Sausage</b>	3.54	22.43	12.67±2.37 <sup>a</sup>	22	73.3	8	26.7

\*According to FAO (2004) regulator limits (< 20 ppb) for meat products. <sup>a-d</sup>Means with different superscripts within the same column significantly ( $p < 0.05$ ) different.

**TABLE 2. Ochratoxin A residues (ppb) in examined beef products and their acceptability\* (n=30 of each)**

Product	Min	Max	Mean±SE	Acceptable sample		Unacceptable sample	
				No	%	No	%
Burger	1.03	3.83	2.64±0.14 <sup>a</sup>	30	100	0	0
Minced beef	0.98	1.91	1.56±0.12 <sup>b</sup>	30	100	0	0
Luncheon	1.45	3.54	2.76±0.43 <sup>a</sup>	30	100	0	0
Basterma	0.72	1.85	1.23±0.65 <sup>c</sup>	30	100	0	0
Kofta	1.15	2.93	1.78±0.74 <sup>b</sup>	30	100	0	0
Sausage	0.97	2.78	2.32±0.57 <sup>a</sup>	30	100	0	0

\*According to FAO (2004) and WHO (2002) regulator limits (< 5 ppb) for meat products. <sup>a-c</sup>Means with different superscripts within the same column significantly ( $p < 0.05$ ) different.

**TABLE 3. Effect of different probiotics on AFL concentration (ppb) experimentally contaminated semi-dry fermented sausage sample**

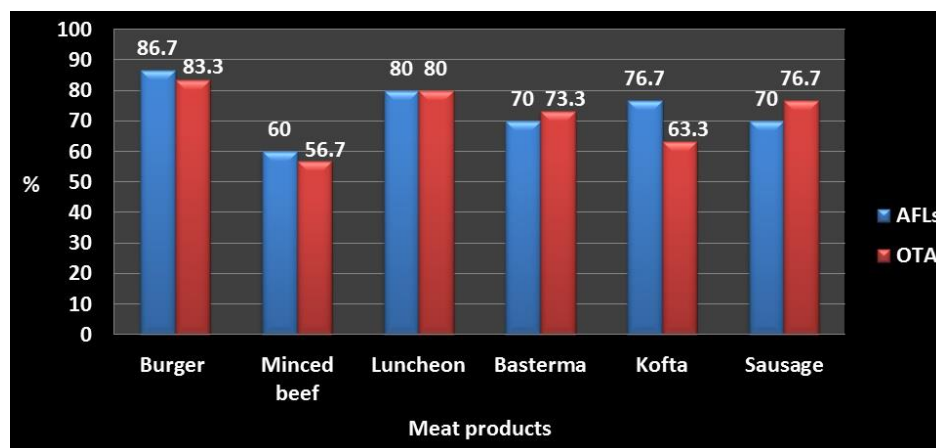
Probiotic group	Mean ± SE of AFL concentration (ppb)			
	Zero	2 <sup>nd</sup> day	5 <sup>th</sup> day	7 <sup>th</sup> day
G1	4.632 <sup>a</sup>	2.976 <sup>c</sup>	1.264 <sup>c</sup>	0.085 <sup>c</sup>
G2	4.597 <sup>a</sup>	3.172 <sup>b</sup>	1.422 <sup>b</sup>	0.774 <sup>b</sup>
G3	4.613 <sup>a</sup>	3.637 <sup>a</sup>	1.638 <sup>a</sup>	1.004 <sup>a</sup>
G4	4.588 <sup>a</sup>	3.287 <sup>b</sup>	1.376 <sup>b</sup>	0.867 <sup>b</sup>

G1: samples treated with *L. acidophilus* (10<sup>7</sup>cfu/g). G2: samples treated with *B. lactis* (10<sup>7</sup>cfu/g). G3: samples treated with *S. cerevisiae* 1%. G4: samples treated with *S. cerevisiae* 2%. Values within the same column have different superscript letters are significantly different at  $p < 0.05$ . Values are expressed as mean ± standard error (SE) of three determinations.

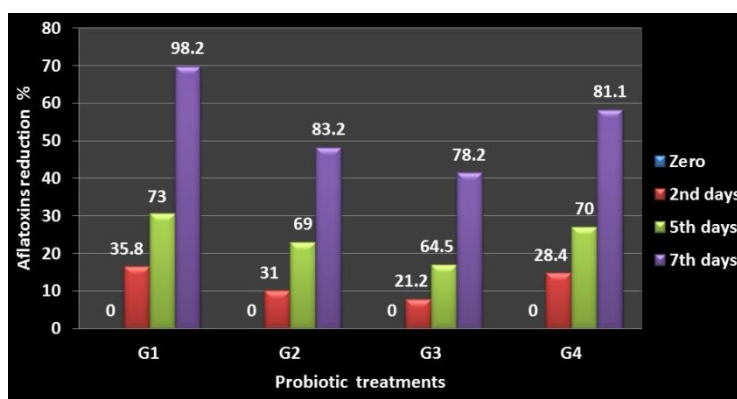
**TABLE 4. Effect of different probiotics on OTA concentration (ppb) experimentally contaminated semi-dry fermented sausage sample**

Group	Mean ± SE of OTA concentration (ppb)			
	Zero	2 <sup>nd</sup> day	5 <sup>th</sup> day	7 <sup>th</sup> day
G1	1.596 <sup>a</sup>	1.332 <sup>b</sup>	1.106 <sup>c</sup>	0.482 <sup>d</sup>
G2	1.648 <sup>a</sup>	1.482 <sup>b</sup>	1.268 <sup>b</sup>	0.854 <sup>b</sup>
G3	1.663 <sup>a</sup>	1.533 <sup>a</sup>	1.378 <sup>a</sup>	0.973 <sup>a</sup>
G4	1.629 <sup>a</sup>	1.387 <sup>b</sup>	1.18 <sup>c</sup>	0.679 <sup>c</sup>

G1: samples treated with *L. acidophilus* (10<sup>7</sup>cfu/g). G2: samples treated with *B. lactis* (10<sup>7</sup>cfu/g). G3: samples treated with *S. cerevisiae* 1%. G4: samples treated with *S. cerevisiae* 2%. Values within the same column have different superscript letters are significantly different at  $p < 0.05$ . Values are expressed as mean ± standard error (SE) of three determinations.

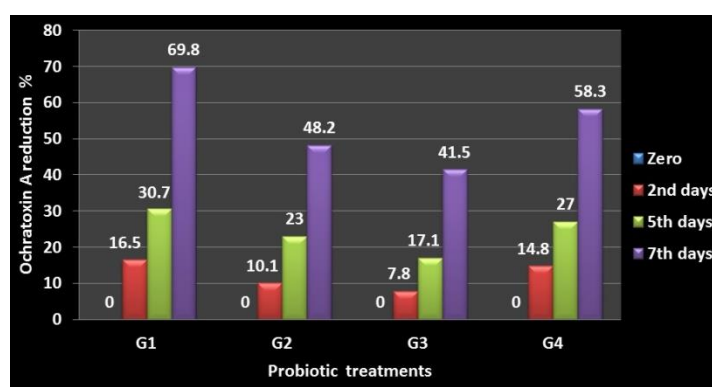
**Fig. 1. Percentage of meat products containing AFTs and OTA residues**





**Fig. 2.** Reduction percentage of AFL concentration after different probiotics treatments.

G1: samples treated with *Lactobacillus acidophilus* ( $10^7$  CFU/g). G2: samples treated with *Bifidobacterium lactis* ( $10^7$  CFU/g). G3: samples treated with *Saccharomyces cerevisiae* 1%. G4: samples treated with *Saccharomyces cerevisiae* 2%. The discrepancy in the meat products sample reviewed was exceedingly substantial ( $p < 0.05$ ) except for G2 and G4.



**Fig. 3.** Reduction percentage of OTA concentration after different probiotics treatments.

G1: samples treated with *Lactobacillus acidophilus* ( $10^7$  CFU/g). G2: samples treated with *Bifidobacterium lactis* ( $10^7$  CFU/g). G3: samples treated with *Saccharomyces cerevisiae* 1%. G4: samples treated with *Saccharomyces cerevisiae* 2%. The reduction of OTA in examined samples is significantly different ( $p < 0.05$ )

## References

- Ibrahim, H., Amin, R., Tolba, K. and Elokke, A. Study on Aflatoxin Residues in some Meat Products and their Control by Probiotics. *Benha Veterinary Medical Journal*, **34**(1), 232-241 (2018).
- Ulusoy, B. H., Hecer, C., Sayiner, S. and Kaya, Y. F. Presence of aflatoxins and ochratoxin A in samarella (tsamarella), a traditional dried-cured meat of Cyprus. *Journal of Food Science and Technology*, **59**, 3002-3009 (2022).
- Pandey, A. K., Samota, M. K., Kumar A., Silva, A. S. and Dubey, N. K. Fungal mycotoxins in food commodities: present status and future concerns. *Frontiers in Sustainable Food System*, **7**, 1162595 (2023).
- Pandey, A. K., Samota, M. K. and Silva, A. S. Mycotoxins along the tea supply chain: a dark side of an ancient and high valued aromatic beverage. *Critical Reviews in Food Science and Nutrition*, **63**, 8672-8697 (2023).
- Perrone, G., Rodriguez, A., Magista, D. and Magan, N. Insights into existing and future fungal and mycotoxin contamination of cured meats. *Current Opinion of Food Science*, **29**, 20-27 (2019).
- Abbas, H. K., Zablotowicz, R. M. and Bruns, H. A. Modeling the colonization of maize by toxigenic and non-toxicogenic *Aspergillus flavus* strains: implications for biological control. *World Mycotoxin Journal*, **1**, 333-340 (2008).
- Karmi M. Detection of Aflatoxins and Ochratoxin A Residues in Meat Products with Amelioration by Probiotics. *Zagazig Veterinary Journal*, **47**, 213-221 (2019).
- Mateus, A. R. S., Barros, S., Pena, A. and Sanches Silva, A. Mycotoxins in pistachios (*Pistacia vera* L.): methods for determination, occurrence, decontamination. *Toxins*, **13**, 682 (2021).
- Smith, C. A., Woloshuk, C. P., Robertson, D. and Payne, G. A. Silencing of the aflatoxin gene cluster in a diploid strain of *Aspergillus flavus* is suppressed by ectopic aflR expression. *Genetics*, **176**, 2077-2086 (2007).



10. Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., Morelli, L., Canani, R. B., Flint, H. J., Salminen, S., Calder, P. C. and Sanders, M. E. The international scientific association for Probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat. Rev. Gastroenterol. Hepatology*, **11**, 506–514 (2014).
11. Wosinska, L., Cotter, P. D., O’Sullivan, O. and Guinane, C. The potential impact of probiotics on the gut microbiome of athletes. *Nutrients*, **11**, 2270 (2019).
12. Markowiak, P. and Sliżewska, K. Effects of probiotics, prebiotics, and synbiotics on human health. *Nutrients*, **9**, 1021 (2017).
13. Kerry, R. G., Patra, J. K., Gouda, S., Park, Y., Shin, H. S. and Das, G. Benefection of probiotics for human health: A review. *Journal of Food and Drug Analysis*, **26**, 927–939 (2018).
14. Najmus, S. N., Arif, S., Afzel, Q., Ahmed, M., Ara, J. and Chaudhry, Q. Impact of discoloration and picking practices of red chilies on aflatoxin levels. *Pakistan Journal of Botany*, **45**(5), 1669–16722 (2013).
15. Baydar, T., Erkekoglu, P., Sipahi, H. and Sahin, G. Aflatoxin B1, M1 and ochratoxin A levels in infant formulae and baby foods marketed in Ankara, Turkey. *Journal of Food and Drug Analysis*, **15**, 89–92 (2007).
16. Maha, M. E., Mahmoud, E., Nagwa, I. M. K. and Mohamed, K. R. Studies on contamination of dairy products by aflatoxin M1 and its control by probiotics. *Journal of Global Biosciences*, **4**(1), 1294–1312 (2015).
17. Emara, M., Ezzat, G., Mohamed, M., Yassien, N. and Mansour, N. Effect of Moringa oleifera Aqueous Extracts on the Physicochemical Characteristics, Microbiological Quality and Biogenic Amines of Semi-dry Fermented Sausage. *International Journal of Veterinary Science*, **9**, 285–290 (2020).
18. SAS (2008). SAS / STAT User’s guide Release 6.12 Edition. Cary NC, USA: SAS Inst. Inc., (2008).
19. Algammal, A., Elsayed, M., Hashem, H., Ramadan, H., Sherabah, N., Eldiasty, E., Abbas, S. and Hetta, H. Molecular and HPLC-based approaches for detection of aflatoxin B1 and ochratoxin A released from toxigenic Aspergillus species in processed meat. *BMC Microbiology*, **21**(1), 82 (2021).
20. da Rocha, M. E., Freire, F. C., Maia, F. E., Guedes, M. I. and Rondina, D. Mycotoxins and their effects on human and animal health. *Food Control*, **36**(1), 159–65 (2014).
21. Morshdy, A. E. M., Abdelhameed, R. H., Tharwat, A. E., Darwish, W. and Ahmed, N. A. Content and Health Risk Assessment of Total Aflatoxins in the Retailed Beef Luncheon, Sausage, and Pasterma in Zagazig City, Egypt. *Journal of Advanced Veterinary Research*, **13**(3), 479–482 (2023).
22. Elbarbary, N. K., Karmi, M., Abdallah, M. M., Abdel-Motaal, F. F. and Maky, M. A. HPLC Detection of Aflatoxin in Meat, Poultry, and Fish and their Products and Detoxification by Gamma Radiation. *Journal of Advanced Veterinary Research*, **13**(3), 492–500 (2023).
23. FAO. Worldwide regulation for mycotoxin in food and feed in 2003. Rome, 2004. FAO. Food and Nutrition P.81, (2004).
24. Elzupir, A. O. and Abdulkhair, B. Y. Health risk from aflatoxins in processed meat products in Riyadh, KSA. *Toxicol*, **181**, 1–5 (2020).
25. Kademi, H. I., Baba, I. A. and Saad, F. T. Modelling the dynamics of toxicity associated with aflatoxins in foods and feeds. *Toxicology Reports*, **4**, 358–363 (2017).
26. Yang, C., Song, G. and Lim, W. Effects of mycotoxin-contaminated feed on farm animals. *Journal of Hazard Materials*, **389**, 122087 (2020).
27. Cinar, A. and Onbaşı, E. Mycotoxins: the hidden danger in foods. In: Mycotoxins and food safety: IntechOpen.5 Princes Gate Court, London, SW7 2QJ, UK. 2019.
28. WHO. Technical report series. Evaluation of certain mycotoxins in food. Fifty sixth report of the joint FAO/WHO Expert committee on food additive – Geneva. (2002).
29. Abd-Elghany, S. M. and Sallam, K. I. Rapid determination of total aflatoxins and ochratoxins A in meat products by immuno-affinity fluorimetry. *Food Chemistry*, **179**, 253–256 (2015).
30. Zdravec, M., Vahčić, N., Brnić, D., Markov, K., Frece, J., Beck, R. and Pleadin, J. A study of surface moulds and mycotoxins in Croatian traditional dry-cured meat products. *International Journal of Food Microbiology*, **317**, 108459 (2020).
31. Pop, O. L., Suharschi, R. and Gabbianelli, R. Biodetoxification and Protective Properties of Probiotics. *Microorganisms*, **10**, 1278 (2022).
32. Martínez, B., Rodríguez, A., Kulakauskas, S. and Chapot-Chartier, M. P. Cell wall homeostasis in lactic acid bacteria: Threats and defences. *FEMS Microbiology Reviews*, **44**, 538–564 (2020).
33. Hori, T., Matsuda, K. and Oishi, K. Probiotics: A dietary factor to modulate the gut microbiome, host immune system, and gut-brain interaction. *Microorganisms*, **8**, 1401 (2020).
34. Haskard, C. A., El-Nezami, H. S., Kankaanpää, P. E., Salminen, S. and Ahokas, J. T. Surface Binding of Aflatoxin by Lactic Acid Bacteria. *American Society for Microbiology, Applied And Environmental Microbiology*, **67**, 3086 (2001).
35. Ghazvini, R. D., Kouhsari, E., Zibafar, E., Hashemi, S. J., Amini, A. and Niknejad, F. Antifungal Activity and Aflatoxin Degradation of Bifidobacterium Bifidum and Lactobacillus Fermentum Against Toxigenic Aspergillus Parasiticus. *Open Microbiology Journal*, **10**, 197–201 (2016).
36. O’Toole, P. W., Marchesi, J. R. and Hill, C. Next-generation probiotics: the spectrum from probiotics to live biotherapeutics. *Nature Microbiology*, **2**, 17057 (2017).

## تقييم فعالية البروبيوتيك ضد تهديدات الميكوتوكسين في نقائق لحم البقر المخمرة شبه الجافة

نادي خيري البربري<sup>1\*</sup>، اشرف عبدالمالك<sup>2,3</sup>، وجية صبحي درويش<sup>4</sup>، سهيلة فتحي الهواري<sup>5</sup>،  
نيفين منير عبدالمطلب<sup>6</sup>، مروة عبدالسيد علي<sup>7</sup> و محمد قرشي دندراوي<sup>8</sup>

<sup>1</sup>قسم الرقابة الصحية على الأغذية، كلية الطب البيطري، جامعة اسوان ، مصر.

<sup>2</sup>قسم صحة وسلامة وتكنولوجيا الأغذية، كلية الطب البيطري، جامعة اسيوط ، مصر.

<sup>3</sup>كلية الطب البيطري، جامعة بدر ، اسيوط ، مصر.

<sup>4</sup>قسم صحة وسلامة وتكنولوجيا الأغذية، كلية الطب البيطري، جامعة الزقازيق ، مصر.

<sup>5</sup>قسم الأحياء، كلية العلوم، جامعة جازان، المملكة العربية السعودية.

<sup>6</sup>قسم تكنولوجيا الأغذية، معهد بحوث زراعة الأراضي القاحلة، مدينة البحوث العلمية والتطبيقات التكنولوجية، مدينة برج العرب الجديد، مصر.

<sup>7</sup>أخصائي ميكروبيولوجي، معمل الفيوم للتحليلات الميكروبيولوجية والمناعية، الفيوم ، مصر.

<sup>8</sup>قسم الرقابة الصحية على الأغذية، كلية الطب البيطري، جامعة جنوب الوادي، مصر.

\*المؤلف المراسل: نادي خيري البربري [nadykhairy@vet.aswu.edu.eg](mailto:nadykhairy@vet.aswu.edu.eg)

### الملخص

يشكل تعرض الطعام للسموم الفطرية مصدر قلق كبير لمسؤولي الصحة العامة والسلطات الرقابية على مستوى العالم. يمكن أن يحدث تلوث منتجات اللحوم بالأفلاتوكسين والأوكراتوكسين (أ) في أي مكان على طول عملية الإنتاج، من المزرعة إلى المائدة. الغرض من هذه الدراسة هو النظر في مخاطر الميكوتوكسين في بعض منتجات لحوم البقر ومعرفة تأثير البروبيوتيك المختلفة على الأفلاتوكسين والأوكراتوكسين (أ) في نقائق لحم البقر المخمرة جزئياً والتي تلوثت في تجربة ثم تم تخزينها لمدة سبعة أيام. وتوصلت الدراسة إلى وجود بقايا الأفلاتوكسين في 86.7% و 60% و 80% و 70% و 76.7% و 70% من منتجات اللحوم قيد الدراسة، بينما كانت بقايا الأوكراتوكسين (أ) موجودة في 83.3% و 56.7% و 80% و 73.3% و 63.3% و 76.7% من البرجر واللحم المفروم واللانسون والبسطرمة والكفتة والسجق على التوالي. وكان البرجر  $2.62 \pm 13.89$  جزء في المليار) والسجق  $2.37 \pm 12.67$  جزء في المليار) أعلى بقايا الأفلاتوكسين، يليهما الكفتة  $11.38 \pm 2.15$  جزء في المليار) واللانسون  $2.72 \pm 11.26$  جزء في المليار). وسجلت البسطرمة  $1.85 \pm 3.31$  جزء في المليار) واللحم المفروم  $1.55 \pm 5.47$  جزء في المليار) أقل القيم. وسجلت عينات اللانسون أعلى نسبة من بقايا الأوكراتوكسين (أ)  $0.43 \pm 2.76$  جزء في المليار)، يليها البرجر  $0.14 \pm 2.64$  جزء في المليار)، والسجق  $0.57 \pm 2.32$  جزء في المليار)، والكفتة  $0.74 \pm 1.78$  جزء في المليار)، بينما سجلت البسطرمة  $0.65 \pm 1.23$  جزء في المليار) واللحم المفروم  $0.12 \pm 1.56$  جزء في المليار) أقل التركيزات. وتكشف نتائج التحقيق أن مستويات الأفلاتوكسين في بعض العينات المفحوصة تجاوزت الحدود القانونية (أقل من 20 جزء في المليار)، في حين جاءت مستويات الأوكراتوكسين (أ) ضمن النطاق المقبول (أقل من 5 جزء في المليار). تظهر البيانات وجود علاقة إيجابية بين استخدام البروبيوتيك وتقليل الأفلاتوكسين والأوكراتوكسين (أ) في جميع العينات المدروسة. إن إضافة *Lactobacillus acidophilus* و 2% *Saccharomyces cerevisiae* هي المسؤولة بشكل أساسي عن تقليل الأفلاتوكسين والأوكراتوكسين (أ) أثناء التخزين. يمكن أن نستنتج أن البروبيوتيك يقلل بشكل كبير من خطر السموم الفطرية في منتجات اللحوم للمستهلكين.

**الكلمات الدالة:** الأفلاتوكسينات، إزالة السموم، منتجات اللحوم، البروبيوتيك، الأوكراتوكسين